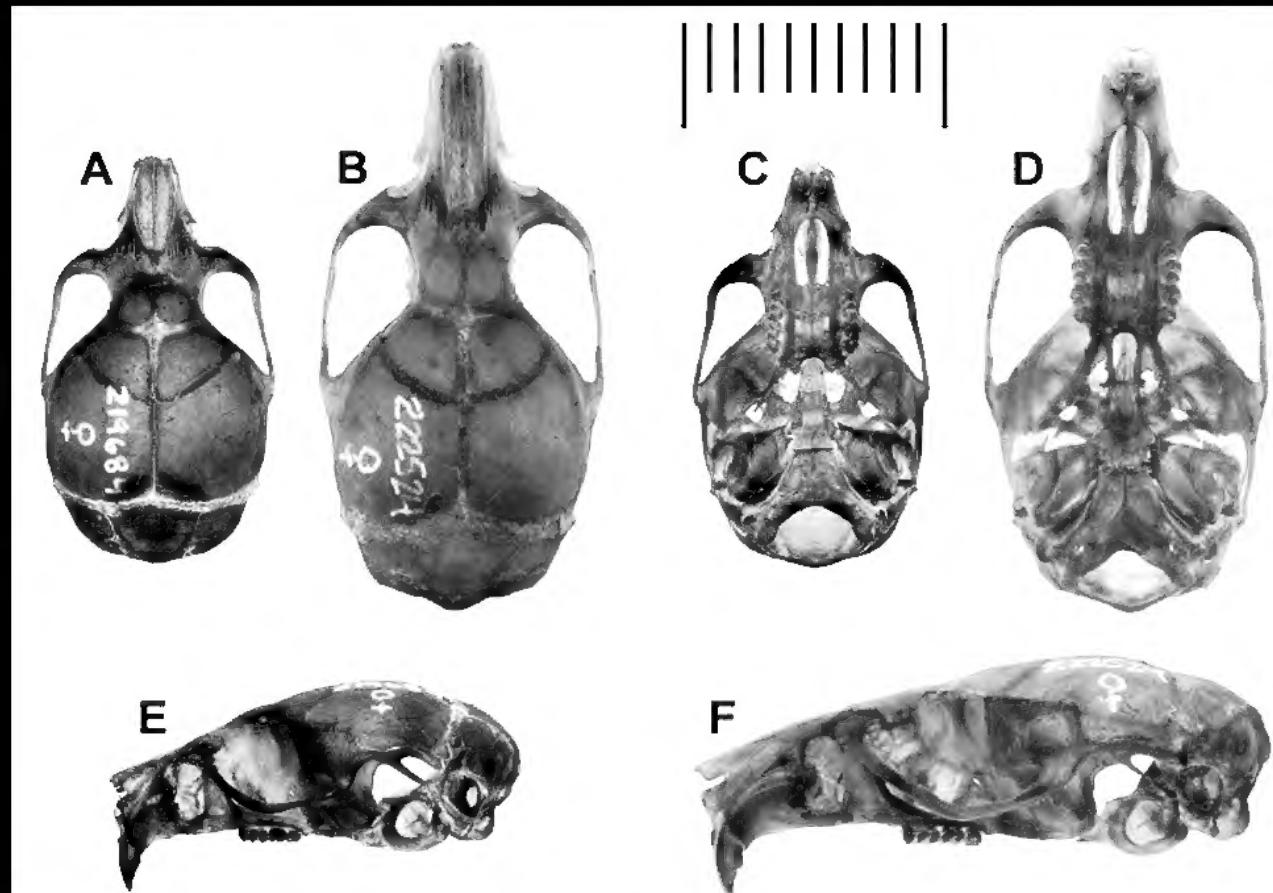


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## Research article

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# New records and an updated list of reptiles from Ba Vi National Park, Vietnam

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**Abstract.** We report eight new records of reptiles from Ba Vi National Park, Hanoi, Vietnam: *Gekko palmatus*, *Boiga guangxiensis*, *Gonyosoma prasinum*, *Lycodon futsingensis*, *L. ruhstrati abditus*, *Opisthotropis lateralis*, *Hebius chamaensis*, and *Pareas hamptoni*. Our findings bring the total number of reptiles recorded from Ba Vi National Park to 50.

**Key words.** Reptiles, new records, distribution, Ba Vi National Park.

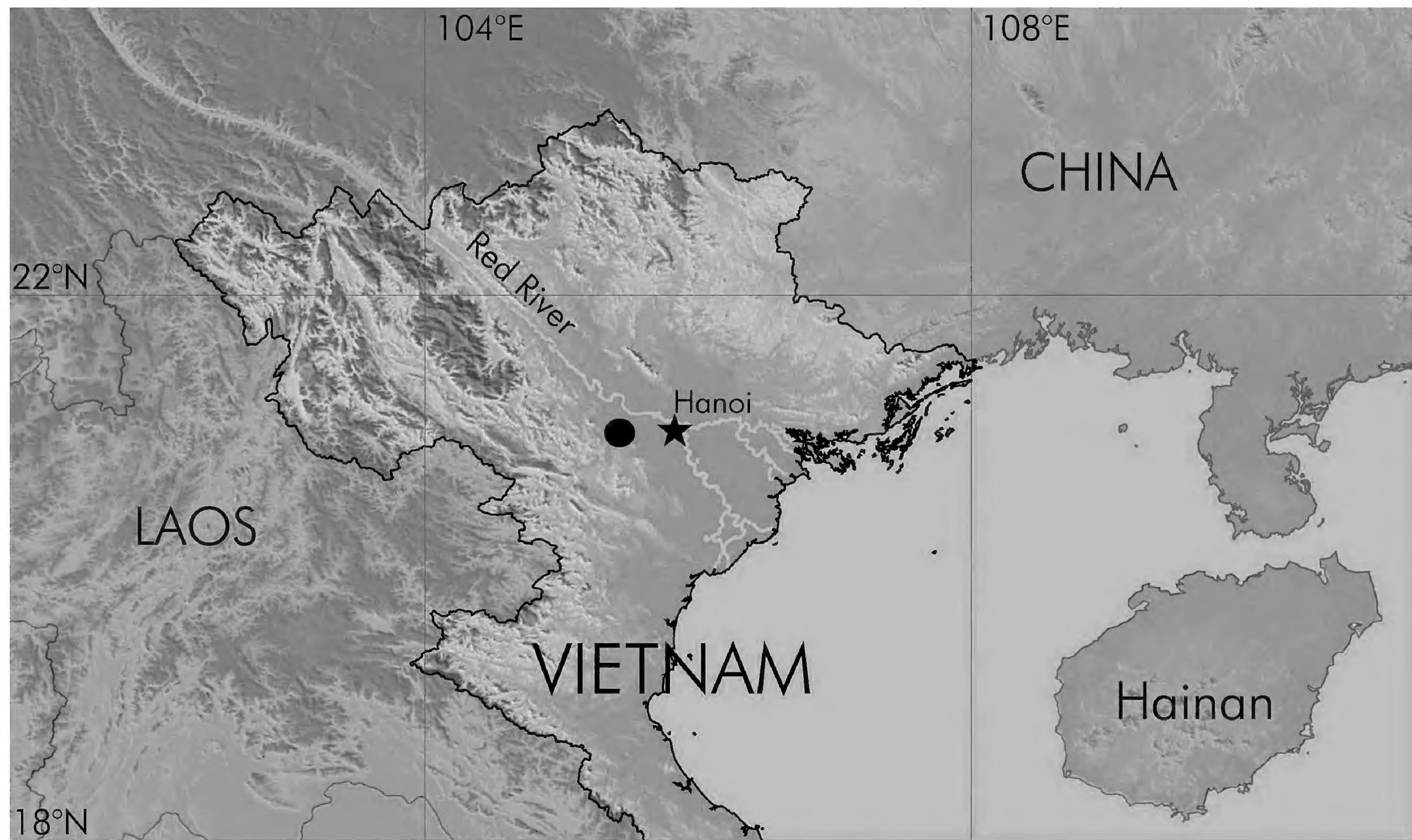
## INTRODUCTION

Ba Vi National Park (NP) is located in Ba Vi District of Hanoi and Luong Son and Ky Son districts of Hoa Binh Province, with a total area of 12,023 ha. There are three high peaks in the national park: the highest is Dinh Vua at 1,296 m above sea level (a.s.l.), followed by Tan Vien at 1,226 m a.s.l. and Ngoc Hoa at 1,120 m a.s.l. (Fig. 1). The Ba Vi NP is characterized by evergreen forest and mixed forest of coniferous and broadleaf at elevations above 600 m a.s.l. (Tran et al. 2001). In Ba Vi District, previous studies documented a total of 42 species of reptiles and most of them were recorded from Ba Vi National Park (Nguyen et al. 2009). As a result of recent herpetological field surveys we herein present eight new records of reptiles from Ba Vi National Park.

## MATERIAL AND METHODS

Field surveys were conducted in the Ba Vi NP by Vinh Q. Luu, Oanh V. Lo, Ngoan V. Ha, Huy Q. Tran, Tuong S. Dinh, Linh K. Luong, Nghia V. Ha and Le D. Phan

(hereafter V. Q. Luu et al.) in July, October and November 2018. Survey sites were selected at elevations ranging from 400 to 800 m a.s.l. Three survey transects were set up at three sites in the mixed forest of coniferous and broadleaf plants: the Transect 1 along Ngoc Hoa stream at elevations of ca. 400 m a.s.l., the Transect 2 in the former French Camping area at elevations of 600 m a.s.l., and the Transect 3 in the forest near Ruins of Old French Church at elevations of ca. 800 m a.s.l. Specimens were collected by hand or using a snake hook. Specimens were euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons, 2002), fixed in 80% ethanol for four to six hours, then later transferred to 70% ethanol for permanent storage. The specimens were subsequently deposited in the collection of the Vietnam National University of Forestry (VNUF), Hanoi, Vietnam. Taxonomic identification mainly followed Smith (1943), Ziegler et al. (2007, 2008, 2018), Nguyen et al. (2009), Vogel et al. (2009), Luu et al. (2013), Hecht et al. (2013), Do et al. (2016), Gawor et al. (2016), Nguyen et al. (2016), Le et al. (2018), and Pham et al. (2018).



**Fig. 1.** Map of Ba Vi National Park (black circle) in Hanoi City, northern Vietnam.

## Abbreviations

SVL = snout-vent length: from tip of snout to anterior margin of cloaca  
 TaL = tail length: from posterior margin of cloaca to tip of tail  
 HL = head length: from the tip of snout to back of mandible  
 HW = head width: the widest portion of the head. Bilateral scale counts were given as left/right.

## RESULTS

### Family Gekkonidae Gray, 1825

#### *Gekko palmatus* Boulenger, 1907

Palm Gecko / Tắc kè chân vịt (Fig. 2a)

**Specimens examined** (n=3). One adult male VNUF R.2018.19 (field number: BV.18.19) collected on 29 July 2018 in the fire protection and prevention station (21°04'529N/105°22'719"E, at an elevation of 400 m a.s.l.) and two adult females VNUF R.2018.12 (field number: BV.18.12) collected on 26 July 2018 in the ancient church area (21°04'409N/105°21'902"E, at an elevation of 800 m a.s.l.) and VNUF R.2018.09 (field number: BV.18.09) collected on 10 July 2018 in Ngoc

Hoa cave (21°05'159"N/105°22'701"E, at an elevation of 400 m a.s.l.) by V. Q. Luu et al.

**Morphological characters.** The specimens from Ba Vi NP agreed well with the description of Rösler et al. (2011): SVL 71.3 mm (male) and 72.2–74.2 mm (females), TaL 12.9–78.6 mm (the female VNUF R.2018.12 with lost tail); head longer than wide (HL 21.5–37.8 mm, HW 13.4–14.6 mm in females; HL 19.9 mm, HW 14.7 mm in the male); rostral wider than high (RW 2.5–3.5 mm, RH 0.9–1.4 mm in females; RW 2.2 mm, RH 1.2 mm in the male); nostril in contact with rostral; interorbital 31–33; preorbitals 18–19; supralabials 11–13; infralabials 10–11; scale rows at midbody 121–125; tubercle rows at midbody 7–10 in the female and 8 in the male; tubercles absent on dorsal surface of limbs; ventral scale rows at midbody 41–50; scales in a line from mental to the front of cloacal slit 166 in the male, 171–176 in females; subdigital lamellae under first finger 11–12, under fourth finger 12–15, under first toe 12–13, under fourth toe 13–14; precloacal pores 26 in the male, absent in females; postcloacal tubercle 1/1.

**Coloration in life.** Dorsal surface of head, body and tail greyish with dark blotches on head and nape; 4–6 larger blotches on dorsum; flanks and limbs with small light spots; dorsal surface of tail of the female VNUF R.2018.12 with eight light bands and of the female

VNUF R.2018.09 with 10 light bands, ventral side of tail yellowish cream with black dots.

**Ecological notes.** The adult female VNUF R.2018.12 was found on the wall of the ancient church at 22:13. Temperature was 23.9 °C and humidity was 71%. The adult male was collected in a house at 24:00 while crawling on the wall. The female VNUF R.2018.09 was found on the roadside cliff at 20:43 with the relative temperature being 26.5 °C and humidity about 55%.

**Distribution.** In Vietnam, this species was recorded from Lao Cai Province in the North southwards to Quang Binh Province (Nguyen et al. 2009, Hecht et al. 2013, Gawor et al. 2016, Pham et al. 2018; Uetz et al. 2019). This is the first record of *Gekko palmatus* for Ba Vi NP and for Hanoi City.

**Remarks.** The specimens from Ba Vi differ from the description of Rösler et al. (2011) by having fewer scale rows at midbody (121–125 *versus* 139–156).

#### Family Colubridae Oppel, 1811

##### *Boiga guangxiensis* (Wen, 1998)

Guangxi Cat Snake / Rắn rào quang tây (Fig. 2b)

**Specimen examined.** One adult female, VNUF R.2018.7 (field number: BV.18.7) collected on 6 October 2018 in the ancient church area (21°04'421"N/105°21'865"E, at an elevation of 801 m a.s.l.) by V. Q. Luu et al.

**Morphological characters.** The specimen from Ba Vi NP agreed well with the description of Ziegler et al. (2007): SVL 1290 mm, TaL 435 mm; head longer than wide (HL 31 mm, HW 21.1 mm), distinct from neck; pupil round; internasal shorter than prefrontal; loreal 1/1; preocular 1/1; postoculars 2/2; anterior temporals 2/2, posterior temporals 2/3; supralabials 8/8, third to fifth in contact with the eye, eighth largest; infralabials 13/12, third to fifth in contact with the eye, eighth largest; first to fourth (left side) and first to third (right side) bordering chin shields; dorsal scale rows 23–21–15, smooth; ventrals 270; cloacal single; subcaudals 145, divided.

**Coloration in life.** Dorsum pale brown, with irregular black cross-bars, venter greyish-white.

**Ecological notes.** The specimen was found at 20:09 while crawling on the forest ground near a tourist road. Distribution. In Vietnam, this species has been recorded from Lao Cai Province southwards to Dong Nai and Tay Ninh provinces (Nguyen et al. 2009; Do et al. 2016; Phan et al. 2018). This is the first record of *Boiga guangxiensis* for Ba Vi NP and for Hanoi City. Elsewhere, this species is known from southern China, Cambodia and Laos (Nguyen et al. 2009; Neang et al. 2017; Uetz et al. 2019).

#### *Gonyosoma prasinum* (Blyth, 1854)

Green bush ratsnake / Rắn sọc xanh (Fig. 2c)

**Specimen examined.** One adult female, VNUF R.2018.30 (field number: BV3.18.10) collected on 15 November 2018 in the ancient church area (21°04'363"N/105°21'886"E at an elevation of 805 m a.s.l.) by V. Q. Luu et al.

**Morphological characters.** The specimen from Ba Vi NP agreed with the description of Hecht et al. (2013), SVL 820 mm, Tal 330 mm; head longer than wide (HL 30.9 mm, HW 16 mm), head distinct from neck; pupil round; internasal suture 1.6 mm; prefrontal suture 4.3 mm; loreal 1/1, not touching the eye; preocular 1/1; postoculars 2/2; anterior temporals 2/2, posterior temporals 2/2; supralabials 9/9, fourth to sixth in contact with the eye, eighth largest; infralabials 10/10; first to fourth (both sides) bordering chin shields; dorsal scale rows 21–19–15, keeled; ventrals 197; cloacal divided; subcaudals 106, divided.

**Coloration in life.** Dorsal surface of head, back and tail green. The upper lip, lower throat, venter and lower surface of tail light green. Each side of the ventrolateral fold has a pale whitish stripe that runs from the neck to cloaca.

**Ecological notes.** The specimen was found in the evening on the wall of the ancient church.

**Distribution.** In Vietnam, this species was reported from Lao Cai Province in the North southwards to Gia Lai Province (Nguyen et al. 2009; Hecht et al. 2013; Le et al. 2018). This is the first record of *Gonyosoma prasinum* for Ba Vi NP and for Hanoi City. Elsewhere, the species is known from India, Southern China, Myanmar, Laos, Thailand, Malaysia (Nguyen et al. 2009; Uetz et al. 2019).

**Remarks.** The specimen from Ba Vi differs from those in the descriptions of Smith (1943) and Hecht et al. (2013) by having more dorsal scale rows at neck (21–19–15 *versus* 19–19–15).

#### *Hebius chapaensis* (Bourret, 1934)

Sapa Flat-nosed Snake / Rắn bình mũi sa pa (Fig. 2d)

**Specimen examined.** One adult female, VNUF R.2018.8 (field number: BV.18.8) collected on 15 November 2018 (21°04'821"N/105°22'190"E, at an elevation of 402 m a.s.l.), by V. Q. Luu et al.

**Morphological characters.** The specimen from Ba Vi NP agreed with the description of Le et al. (2018): SVL 440 mm, TaL 140 mm; head longer than wide (HL 13.6 mm, HW 8.82 mm), distinct from the neck; pupil



**Fig. 2.** Dorsal and dorsolateral views of the eight new records of reptiles from Ba Vi National Park, Hanoi, Vietnam. **a.** *Gekko palmatus* (VNUF R.2018.12). **b.** *Boiga guangxiensis* (VNUF R.2018.7). **c.** *Gonyosoma prasinum* (VNUF R.2018.30). **d.** *Hebius chapaensis* (VNUF R.2018.8). **e.** *Lycodon futsingensis* (VNUF R.2018.5). **f.** *Lycodon ruhstrati abditus* (VNUF R.2018.10). **g.** *Opisthotropis lateralis* (VNUF R.2018.37). **h.** *Pareas hamptoni* (VNUF R.2018.18).

round; loreal 1/1, not touching the eye; preoculars 2/2; postoculars 2/2; anterior temporals 1/1, posterior temporals 2/2; supralabials 9/9, fifth to sixth in contact with the eye, seventh largest; infralabials 10/10, first to fifth (both sides) bordering chin shields; dorsal scale rows 17–17–17, feebly keeled; scales of the outer row enlarged; ventrals 170; cloacal divided; subcaudals 70, divided.

**Coloration in life.** Dorsal surface of head, body and tail blackish grey, with two broader light yellow dorsolateral stripes along the body; ventral surface and lower surface of tail black.

**Ecological notes.** The specimen was found in the evening in a stream while being kept by a crab.

**Distribution.** In Vietnam, this species was reported from Lao Cai, Son La, and Yen Bai provinces. This is the first record of *Hebius chapaensis* for Ba Vi National Park and for Hanoi City. Elsewhere, the species is known from China (Uetz et al. 2019).

**Remarks.** *Pararhabdophis chapaensis* was originally described from Sa Pa, Lao Cai Province by Bourret (1934) and it was considered as a poorly known species, known only from northwestern Vietnam and Yunnan Province of China. This species was recently transferred to the genus *Hebius* by Kizirian et al. (2018).

***Lycodon futsingensis* (Pope, 1928)**  
Futsing Wolf Snake / Rắn khuyết fut-sing (Fig.2e)

**Specimen examined.** One adult male, VNUF R.2018.5 (field number: BV.18.5) collected on 6 October 2018 in the ancient church area (21°04'383"N/105°21'856"E, at an elevation of 800 m a.s.l.) by V. Q. Luu et al.

**Morphological characters.** The specimen from Ba Vi NP agreed well with the description of Vogel et al. (2009): SVL 680 mm, Tal 160 mm; head distinct from neck; pupil round; nasal divided; loreal 1/1, not touching the eye; preocular 1/1; subocular absent; postoculars 2/2; anterior temporals 2/2, posterior temporals 3/3; supralabials 8/8, third to fifth in contact with the eye, sixth largest; infralabials 10/10, first to third (left side) and first to fifth (right side) bordering chin shields; dorsal scale rows 17–17–15, smooth; ventrals 204; cloacal single; subcaudals 74, divided.

**Coloration in life.** Dorsum chocolate brown with 30 white brown bands on the body and 13 bands on the tail. Ventral surface white with dirty brown marbling, dark brown posteriorly.

**Ecological note.** The specimen was found at 20:39 on the forest ground. The relative temperature was about 22.6 °C and the humidity 60%.

**Distribution.** In Vietnam, this species was reported from Lao Cai Province southwards to Da Nang City. This is the first record of *Lycodon futsingensis* for Ba Vi NP and for Hanoi City. Elsewhere, the species is known from China, Laos, Japan, Taiwan, Myanmar (Hecht et al. 2013; Luu et al. 2013; Nguyen et al. 2018; Uetz et al. 2019).

***Lycodon ruhstrati abditus* (Vogel, David, Pauwels, Sunantha, Norval, Hendrix, Vu & Ziegler, 2009)**  
Mountain Wolf Snake / Rắn khuyết đóm (Fig.2f)

**Specimen examined.** One adult male, VNUF R.2018.10 (field number: BV.18.10) collected on 26 July 2018 in the camping area (21°04'526"N/105°22'189"E, at an elevation of 672 m a.s.l.) by V. Q. Luu et al.

**Morphological characters.** The specimen from Ba Vi NP agreed with the description of Vogel et al. (2009), SVL 696 mm, TaL 190 mm; TL 886 mm; body elongate; head moderately distinct from neck, head longer than wide (HL 19.4 mm, HW 12.2 mm) rather flattened; snout projecting anteriorly beyond lower jaw; pupil vertically oval; tail tapered and thin; loreal 1/1; loreal not in contact with eye; supralabials 8/8, third to fifth in contact with the eye, sixth largest; infralabials 9/8, first to fifth (left side) and first to fourth (right side) bordering chin shields; posterior chin shields a little shorter than anterior ones; preocular 1/1; postoculars 2/2; anterior temporals 1/1, posterior temporals 3/2; dorsal scale rows 17–17–15, keeled; ventrals 229; subcaudals 100, divided; cloacal single.

**Coloration in life.** Dorsal surface of body greyish black with 26 cross-bars, including two white bands near the neck and 24 brown bands on body. Ventral surface white with some small brown spots posteriorly. Dorsal surface of tail greyish brown with 16 cream rings, extending towards the lower surface of the tail.

**Ecological notes.** The specimen was found at 21:18 in the shrub near a forest path. The surrounding habitat was secondary forest mixed with bamboos. The relative temperature was 25.1 °C and the humidity was 71%.

**Distribution.** In Vietnam, this species was previously known from Vinh Phuc and Quang Binh provinces. This is the first record of *Lycodon ruhstrati abditus* for Ba Vi NP and for Hanoi City. Elsewhere, this species is known from China and Laos (Vogel et al. 2009; Luu et al. 2013; Uetz et al. 2019).

**Remarks.** The specimen from Ba Vi NP differs from the description of Vogel et al. (2009) by having more ventrals (229 *versus* 214–224) and the first body band starting at ventral 24 (*versus* at ventrals 12–17).

***Opisthotropis lateralis* Boulenger, 1903**

Tonkin Mountain Keelback / Rắn trán bén (Fig. 2g)

**Specimen examined.** one adult male, VNUF R.2018.37 (field number: BV3.18.07) collected on 15 November 2018 (21°04'821"N/105°22'190"E, at an elevation of 400 m a.s.l.) by V. Q. Luu et al.

**Morphological characters.** The specimen from Ba Vi NP agreed with the description of Hecht et al. (2013): SVL 470 mm, TaL 85 mm; head length larger than wide (HL 17.5 mm, HW 10.3 mm), rostral broader than high; internasals paired; prefrontal single; frontal longer than wide, shorter than parietals, twice as broad as supraocular; nostrils directing upwards, in the upper part of single nasal; internasal suture 1 mm; prefrontal suture 2.4 mm; loreal 1/1, not touching the eye; preocular 1/1; postoculars 2/2; anterior temporal 1/1, posterior temporals 2/2; supralabials 9/9, fifth to sixth in contact with the eye, eighth largest; infralabials 10/10, first to fourth (both sides) bordering chin shields; dorsal scale rows 17–17, keeled; ventrals 182; cloacal divided; subcaudals 50, divided.

**Coloration in life.** Dorsal surface of head and body dark greyish brown, ventral surface yellowish white.

**Ecological notes.** The specimen was found at night in a stream after heavy rain.

**Distribution.** In Vietnam, this species was reported from Cao Bang, Lang Son, Vinh Phuc, Quang Ninh, Bac Giang, Hai Duong, and Hoa Binh provinces (Nguyen et al. 2009; Hecht et al. 2013; Gawor et al. 2016). This is the first record of *Opisthotropis lateralis* for Ba Vi National Park and for Hanoi City. Elsewhere, the species is reported from China (Nguyen et al. 2009; Uetz et al. 2019).

**Family Pareatidae Romer, 1956**

***Pareas hamptoni* (Boulenger, 1905)**

Hampton's Slug Snake / Rắn hổ mây ham-ton (Fig. 2h)

**Specimens examined (n=2).** One adult female VNUF R.2018.18 (field number: BV.18.18) collected on 28 July 2018 in the orchid garden (21°04'529"N, 105°22'719"E, at elevation of 719), and one adult male VNUF R.2018.04 (field number: BV.18.04) on 6 October 2018 in the ancient church area (21°04'397"N/105°21'845"E, at an elevation of 400 m a.s.l.) by V. Q. Luu et al.

**Morphological characters.** The specimen from Ba Vi NP agreed with the description of Ziegler et al. (2007); Pham et al. (2018); Nguyen et al. (2018); SVL 453–480 mm, TaL 141–160 mm; body strongly compressed; head distinct from neck; head longer than wide (HL 15.8–17.3 mm, HW 8.7–11.1 mm); nasal undivided; loreal 1/1, not touching the eye; preoculars 2/2; postoculars 2/2; suboculars 2/2, long and slender; anterior temporals 2/2, posterior temporals 3/2; supralabials 8/7; infralabials 8/8; mental groove absent; dorsal scales smooth in the male and feebly keeled in the female; dorsal scale rows 15–15–15; ventrals 188–189; cloacal single; subcaudals 89–100, divided.

**Coloration in life.** Dorsal surface of head and body reddish brown, with vertical black body bands; ventral scales from chin to lower surface of tail orange with black spots in the female, dorsal surface of tail with black stripe.

**Ecological notes.** The female was found at 20:30 on the road in the heavy rain and the male was found at 20:48 on the roadside.

**Distribution.** In Vietnam, this species has been recorded from Lao Cai Province in the North southwards to Lam Dong and Dong Nai provinces (Nguyen et al. 2009; Nguyen et al. 2018; Phan et al. 2018; Le et al. 2018). This is the first record of *P. hamptoni* for Ba Vi NP and for Hanoi City. Elsewhere, the species is known from China, Myanmar, Laos, and Cambodia (Nguyen et al. 2009; Uetz et al. 2019).

**DISCUSSION**

Our new findings of reptiles from Ba Vi National Park bring the total species number of reptiles in this national park to 50 (see Table 1, Appendix). In recent years, some taxonomic changes have been made concerning reptiles in Vietnam. Nguyen et al. (2009) reported *Ophisaurus harti* (Boulenger, 1899) from Ba Vi NP but it was re-identified as *Dopasia ludovici* by Nguyen et al. (2011). In this study, we provide the first record of *Hebius chapaensis* but did not find any *H. khasiensis* (Boulenger, 1890). Therefore, the previous record of *H. khasiensis* in Ba Vi NP needs to be confirmed on the basis of voucher specimens because *H. chapaensis* and *H. khasiensis* are morphologically similar to each other.

In terms of conservation concern, among 50 recorded species of reptiles, five species were listed in the IUCN Red List (2019), eight species were listed in the Vietnam Red Data Book (2007) and three species were listed in the Governmental Decree No. 06 (2019).

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## APPENDIX

**Table 1.** Updated list of reptiles recorded from Ba Vi NP.

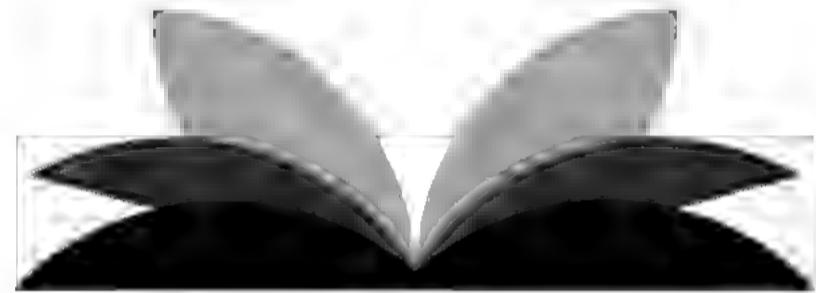
Data sources: 1: Nguyen et al. (2009); 2: This study; 3: Nguyen et al. (2011).  
 Decree 06 (2019) = Governmental Decree No 06/2019/NĐ-CP by the Government of Vietnam on the management of endangered wild flora and fauna. Group IB: prohibited exploitation and use for commercial purpose and Group IIB: limited exploitation and use for commercial purpose; RBVN (2007) = Vietnam Red Data Book. Part I. Animals. Descriptions of nationally endangered species of wild animals. CR = Critically Endangered, EN = Endangered, VU = Vulnerable; IUCN (2019) = The IUCN Red List of Threatened Species. CR = Critically Endangered, EN = Endangered, VU = Vulnerable, LR/nt = Lower Risk/Near Threatened, \* new provincial record; \*\* most probably *Pelodiscus variegatus*, see Farkas et al. (2019).

Species name	Data source	IUCN (2019)	RBVN (2007)	Decree 06 (2019)
<b>REPTILIA</b>				
<b>Squamata</b>				
<b>Sauria</b>				
<b>Agamidae</b>				
<i>Acanthosaura lepidogaster</i> (Cuvier, 1829)	1, 2			
<i>Draco maculatus</i> (Gray, 1845)	1			
<i>Pseudocalotes brevipes</i> (Werner, 1904)	1			
<i>Physignathus cocincinus</i> Cuvier, 1829	1		VU	
<b>Gekkonidae</b>				
<i>Gekko palmatus</i> Boulenger, 1907*	2			
<i>Gekko reevesii</i> (Gray, 1831)	1		VU	
<i>Hemidactylus frenatus</i> Dumérin & Bibron, 1836	1,2			
<b>Scincidae</b>				
<i>Eutropis longicaudatus</i> (Hallowell, 1857)	1			
<i>Tropidophorus baviensis</i> Bourret, 1939	1, 2			
<i>Tropidophorus hainanus</i> Smith, 1923	1			
<b>Anguidae</b>				
<i>Dopasia ludovici</i> (Mocquard, 1905)	1, 2, 3			
<b>Serpentes</b>				
<b>Colubridae</b>				
<i>Ahaetulla prasina</i> (Boie, 1827)	1			
<i>Boiga guangxiensis</i> Wen, 1998*	2			
<i>Boiga multomaculata</i> (Boie, 1827)	1			
<i>Calamaria pavimentata</i> Duméril, Bibron & Duméril, 1854	1			
<i>Calamaria septentrionalis</i> Boulenger, 1890	1, 2		VU	
<i>Coelognathus radiatus</i> (Boie, 1827)	1		VU	
<i>Gonyosoma boulengeri</i> (Mocquard, 1897)	1			
<i>Gonyosoma prasinum</i> (Blyth, 1854)*	2		VU	
<i>Hebius chapaensis</i> (Bourret, 1934)*	2			
<i>Hebius khasiensis</i> (Boulenger, 1890)	1			
<i>Hebius sauteri</i> (Boulenger, 1909)	1			
<i>Lycodon futsingensis</i> (Pope, 1928)*	2			
<i>Lycodon meridionalis</i> (Bourret, 1935)	1, 2			

**Table 1.** Continued

Species name	Data source	IUCN (2019)	RBVN (2007)	Decree 06 (2019)
<i>Lycodon ruhstrati abditus</i> Vogel, David, Pauwels, Sumontha, Norval, Hendrix, Vu & Ziegler, 2009*	2			
<i>Lycodon subcinctus</i> Boie, 1827	1			
<i>Oligodon cinereus</i> (Günther, 1864)	1			
<i>Oligodon eberhardti</i> Pellegrin, 1910	1			
<i>Ptyas korros</i> (Schlegel, 1837)	1		EN	
<i>Ptyas major</i> (Günther, 1858)	1			
<i>Ptyas multicinctus</i> (Roux, 1907)	1, 2			
<i>Ptyas nigromarginata</i> (Blyth, 1854)	1			
<b>Homalopsidae</b>				
<i>Hypsiscopus plumbea</i> (Boie, 1827)	1			
<b>Natricidae</b>				
<i>Opisthotropis lateralis</i> Boulenger, 1903*	2			
<i>Rhabdophis callichroma</i> (Bourret, 1934)	1			
<i>Rhabdophis subminiatus</i> (Schlegel, 1837)	1, 2			
<i>Xenochrophis flavipunctatus</i> (Hallowell, 1860)	1			
<b>Pseudoxenodontidae</b>				
<i>Pseudoxenodon bambusicola</i> Vogt, 1922	1, 2			
<b>Elapidae</b>				
<i>Bungarus fasciatus</i> (Schneider, 1801)	1		EN	
<i>Naja atra</i> Cantor, 1842	1, 2	VU	EN	IIB
<i>Ophiophagus hannah</i> (Cantor, 1836)	1	VU	CR	IB
<i>Sinomicrurus maclellandi</i> (Reinhardt, 1844)	1, 2			
<b>Pareidae</b>				
<i>Pareas hamptoni</i> (Boulenger, 1905)*	2			
<i>Pareas margaritophorus</i> (Jan, 1866)	1			
<b>Viperidae</b>				
<i>Ovophis monticola</i> (Günther, 1864)	1, 2			
<i>Protobothrops mucrosquamatus</i> (Cantor, 1839)	1, 2			
<i>Trimeresurus albolabris</i> Gray, 1842	1, 2			
<b>Testudines</b>				
<b>Geoemydidae</b>				
<i>Cuora mouhotii</i> (Gray, 1862)	1	EN		IIB
<i>Mauremys sinensis</i> (Gray, 1834)	1	EN		
<b>Trionychidae</b>				
<i>Pelodiscus sinensis</i> (Wiegmann, 1835)**	1	VU		

BHL



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## Research article

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# Helminths associated with terrestrial slugs in some parts of Europe

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<sup>3</sup>urn:lsid:zoobank.org:author:9F5DC05E-80CF-4FB2-AAD2-51D243834D80

<sup>4</sup>urn:lsid:zoobank.org:author:A5944069-0F77-4B9F-BE12-7B302385C7E2

<sup>5</sup>urn:lsid:zoobank.org:author:01C01518-F8C6-436A-BA3E-0C73EF95C1C5

**Abstract.** A survey of helminths associated with terrestrial slugs focusing on the invasive *Arion vulgaris* and the native *A. ater* was conducted on populations from France, Germany, Netherlands, Norway and Poland. In total, 648 terrestrial slugs were collected from 18 sample sites, and identified by means of morphological examination, dissection of genitalia and molecular analysis using mitochondrial DNA. In addition to *A. vulgaris* and *A. ater*, also *A. vulgaris/A. rufus* hybrids and *A. ater/A. rufus* hybrids were collected. Helminth species were identified based on morphological features and sequencing of the 18S and ITS rDNA regions. The parasites included four nematode species: *Alloionema appendiculatum*, *Angiostoma* sp., *Phasmarhabditis hermaphrodita*, *Entomelus* sp., two trematode species: *Brachylaima mesostoma*, *Eurytrema* sp., and one cestode (tapeworm) species: *Skrjabinia* sp. *Alloionema appendiculatum* was the most common helminth in the investigated slug populations. Furthermore, we found higher prevalence of trematodes in the invasive *A. vulgaris* compared with the native *A. ater*, while differences in the prevalence for nematodes were not as clear.

**Keywords.** Slugs, Arionidae, helminth parasites, nematodes, trematodes, tapeworm.

## INTRODUCTION

Parasitism plays an important role in the ecology and evolution of terrestrial gastropods (slugs and snails) influencing the evolution of sexual reproduction, life-history traits as well as host resistance leading to host-parasite co-evolution. Parasites have a direct impact on life cycles of their hosts, and the effects of parasites can be modulated by environmental factors. Several studies have been conducted on the impact of climate change on crop pests in relation to natural enemies such as insect predators, parasitoids, pathogenic microorganisms, and helminth parasites (Gerard et al. 2013; Wilson et al. 2015). The presence of helminths in slugs can be influenced by the size, age and the spatial isolation of the host population and by habitat characteristics (Baur & Baur 2005; Gerard et al. 2013; Wilson et al., 2012; Wilson et al. 2015).

Different groups of helminths can be associated with terrestrial gastropods, but slugs as hosts have been given most attention (Ross et al. 2010a; Ross et al. 2010b; Ross

et al. 2016). Currently more than 25,000 species of nematodes have been described, of which around 3,500 are parasitic nematodes of invertebrates (Lazník et al. 2010). Nematodes parasitise both slugs and snails, however slugs are parasitised more frequently and by a greater diversity of nematodes than snails (Mengert 1953). This is because slugs usually inhabit soil, thus increasing their exposure to nematodes (Morand et al. 2004; Ross et al. 2010a). Currently, representatives of eight nematode families are known to be associated with terrestrial slugs: Agfidae, Allaninematidae, Alloionematidae, Angiostomatidae, Cosmocercidae, Diplogasteridae, Mermithidae, and Rhabditidae (particularly the genus *Phasmarhabditis*) (Ross et al. 2017). These families are known to form a number of different relationships with slugs, including parasitic (specialist or generalist), phoretic and necromenic associations (Ross et al. 2010b; Ross et al. 2017). Moreover, trematodes, mites, sporozoa, ciliates, and cestodes have also been described to interact with slugs (Stephenson & Knutson 1966; Baur & Baur 2005).

Terrestrial gastropods are considered to be one of the most successful and diverse animal groups in terrestrial ecosystems (Barker 2001). Many of them have become invasive species in the context of expanding their range of distribution and generating economic damages (e.g., *Lissachatina fulica* Bowdich, 1822 and *Deroceras reticulatum* Müller, 1774) (Hammond & Byers 2002; Ross et al. 2010a). One of the 100 most invasive species in Europe is *Arion vulgaris* Moquin-Tandon, 1855, commonly known as the Spanish slug. It has probably been unintentionally introduced into new habitats via plant matter, packaging, and waste materials (Kozłowski 2007; Hatteland et al. 2013; Zajac et al. 2017). It is a major defoliator of plants and causes severe damage in orchards and gardens as well as in crops (Gren et al. 2009). This slug has been assumed to originate from the Iberian Peninsula and spread into Central Europe in the 1950s (Frank et al. 2002), although recent studies suggest a more northern origin, possibly in France (Hatteland et al. 2015; Zemanova et al. 2016). Monitoring the spread of *A. vulgaris* is difficult because the pest is morphologically similar to the other closely related, large arionids (*A. ater* Linnaeus, 1758; *A. rufus* Linnaeus, 1758; *A. magnus* Torres Minguez, 1923; *A. lusitanicus* Mabille, 1868; *A. flagellus* Collinge, 1893) that occur in Europe. *Arion ater*, *A. rufus* and *A. vulgaris* can hybridise with each other (Roth et al. 2012; Dreijers et al. 2013) and introgression has readily been shown, especially between *A. ater* and *A. rufus* (Hatteland et al. 2015; Zemanova et al. 2017). Moreover, *A. vulgaris* may outcompete native slug species because of its large size and high population densities (Frank 2003). Native slugs like *A. ater* and *A. rufus* have been observed to decline and/or disappear in areas colonized by invasive slugs such as *A. vulgaris* in continental Europe (Falkner 1990) and Scandinavia (B. A. Hatteland pers. obs.) and a similar pattern seems to occur where *A. flagellus* has been introduced in Britain (Davies 1987).

There are numerous hypotheses regarding what makes species invasive. Release from natural enemies is regarded to be an important factor supporting invasiveness by many organisms (Torchin et al. 2003). The enemy release hypothesis (ERH) states that the lack of natural enemies in an invader's introduced range influences its abundance or impact (e.g., estimated using individual size, population abundance, or propensity to displace native species) (Torchin et al. 2003; Colautti et al. 2004). Torchin et al. (2003) studied parasite burdens in 26 species of invasive animals and found that most of them had fewer parasites in their introduced areas compared with their home ranges. Indeed, it is less likely that hosts will spread parasites into their introduced range since introduced populations often originate from relatively small subsets of native populations (and sometimes from uninfected life-history stages) (Torchin et al. 2003). However, recent studies have shown that *A. vulgaris* hosts a range of parasites in introduced areas as well as relatively high parasitic loads

compared with other native slug species (Ross et al. 2010a; Ross et al. 2016).

This study describes results on the diversity and distribution of helminths associated with *A. vulgaris* and *A. ater* in Europe, i.e., France, Germany, Netherlands, Norway and Poland.

## MATERIAL AND METHODS

### Collection and identification of slugs and helminths

Slugs were collected from 18 sites in Europe (France, Germany, Netherlands, Norway, Poland) in 2015 and 2016 from late August to October (Table 1). Sites were selected based on information from local growers and gardeners as well as advisory services in the region regarding the presence of slugs. At each site more than 10 slugs were collected and sent to the Institute of Environmental Sciences (Jagiellonian University, Kraków, Poland) and Norwegian Institute of Bioeconomy Research (NIBIO, Ås, Norway). Slugs were identified by means of morphological examination guided by von Proschwitz (2009), dissection of their genitalia and molecular analysis using a fragment of mitochondrial cytochrome c oxidase subunit I (COI, mtDNA). The main features of the genitalia in *A. vulgaris* are the small atrium, almost symmetrical, one-partite, bursa copulatrix oval and a free oviduct with a short, thin posterior end and a thick, rapidly expanding anterior end with a large, asymmetric ligula inside (Wiktor 2004). The main characteristics of *A. ater* are the atrium and vagina considerably narrower than spermatheca, oviduct narrow and the spermatheca spherical (Welter-Schultes 2012). During the dissection of slugs, a piece of tissue was taken for DNA extraction and preserved in 96% ethanol at -80°C.

Slugs were checked for potential helminths, which were identified using a combination of morphological and molecular techniques. The helminths were first classified morphologically as nematodes or trematodes under a stereomicroscope Olympus SZX10. For purposes of molecular identification, all helminth samples (i.e., adults, juveniles and cysts) were transferred to Eppendorf tubes containing 70% ethanol. Each helminth was introduced to a separate Eppendorf tube.

### DNA isolation, amplification and sequencing

DNA extraction of slugs was performed using a commercial DNA extraction kit (NucleoSpin® Tissue, Macherey-Nagel, Düren, Germany), which uses a proteinase K to digest proteins within cell membranes and columns with silica membranes and buffers for cleaning extracted DNA. PCR reactions were performed to obtain a fragment of mitochondrial DNA with LCO1490/HC02198 primers (Folmer et al. 1994). A PCR reaction was per-

formed in a reaction mixture of 20 µl per each sample and consisted of 3 µl of template DNA, 0.6 µl of each primer, 2 µl of 10× buffer, 13 µl of ddH<sub>2</sub>O, 0.6 µl of 20 mM dNTP and 0.2 µl of DreamTaq™ DNA Polymerase (Thermo Fisher Scientific Inc., MA, USA). PCR conditions included 5 min initial denaturation at 94°C and then 1 min denaturation at 94°C, 1 min 30 s annealing at 45°C and 1 min 30 s elongation at 72°C for 5 cycles and then 1 min denaturation at 94°C, 1 min 30 s annealing at 50°C and 1 min elongation at 72°C for 35 cycles followed by a final elongation step for 5 min at 72°C. A volume of 5 µl sample of PCR product was run on a 1.0% agarose gel for 30 min at 100 V to check DNA quality. PCR products were cleaned up by commercial kit (NucleoSpin® Gel and PCR Clean-up, Macherey-Nagel, Düren, Germany). The sequencing reaction was performed in a reaction mixture of 10 µl per each sample and consisted of 2 µl of template DNA, 5.85 µl of ddH<sub>2</sub>O, 0.15 µl of primer, 1 µl of 5× buffer and 1 µl of Terminator (BrightDye® Terminator Cycle Sequencing Kit, MCLAB, South San Francisco, USA). Sequencing products were cleaned by using a kit to remove terminators after sequencing reactions (ExTerminator, A&A Biotechnology, Gdynia, Poland). The sequencing reactions were performed in Molecular Ecology Lab, Institute of Environmental Sciences (Jagiellonian University, Kraków, Poland).

The genomic DNA of helminths was isolated with a QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) according to the protocol provided by the manufacturer. DNA concentration and its purity were measured using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). Polymerase chain reactions (PCR) were performed on the partial 18S and ITS rDNA regions. For nematodes, the ITS region was amplified using two sets of primers, i.e. 18S/26S (Vrain et al., 1992), and N93/N94 (Nadler et al. 2005). Amplification of the 18S region was done with 24F/18P primers (Blaxter et al. 1998). DNA of trematodes and cestodes was amplified using 3S/A28 primers for ITS2 region (Bowles et al. 1995). PCR were performed in a reaction mixture of 20 µl per each sample and consisted of 1 µl of template DNA (100 ng of template DNA in 1 µl volume), 1 µl of each forward and reverse primer (0.5 µM), 10 µl of 2× DreamTaq™ Master Mix (Thermo Fisher Scientific Inc., MA, USA) and 7 µl of sterile distilled water. The PCR amplifications were performed as described in Nermut' et al. (2015) for the 18S/26S primers, Nadler et al. (2005) for N93/N94 primers, Ross et al. (2010b) for 24F/18P, and Prasad et al. (2007) for 3S/A28. A volume of 5 µl sample of PCR product was run on a 1.5% agarose gel for 30 min at 100 V to check DNA quality. For rDNA partial sequences, PCR products were sequenced with primers used for PCR reactions. Sequencing was performed by Genomed (Warsaw, Poland). Contigs assembled were determined using BioEdit version 7.1.3.0 (Hall 1999).

## Phylogenetic analysis

The molecular phylogenetic status of helminths was determined using BioEdit version 7.1.3.0 for multiple sequence alignments (Hall, 1999). Multiple nucleotide sequence alignments were generated using also other sequences deposited in GenBank showing the highest similarity to the sequence of examined helminths (Appendix I). The species names and GenBank accession numbers of the sequences compared to the analyzed helminths are shown in the phylogenograms. Phylogenetic trees were generated using MEGA X (Kumar et al. 2018) by maximum likelihood (ML) and neighbour-joining (NJ) algorithms. The base substitution model was determined for the 18S and ITS using MEGA X under the Bayesian Information Criterion. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Bootstrap values above 60% were considered. Branch lengths indicate evolutionary rates expressed as the number of base differences per site.

## Statistical analyses of prevalence

A comparison of parasite prevalence was carried out between the invasive *A. vulgaris* and the native *A. ater*. Hybrid slugs were not included since the number of specimens was low. Generalized linear models (GLMs) with binomial distribution were used to test possible differences in presence/absence of nematodes and trematodes, respectively. All specimens from all investigated populations were included in the data set. Locality was used as an explanatory factor in addition to slug species to test the prevalence of the two different parasite groups. Statistical analyses were performed in the software R (R Core Team 2017).

## RESULTS

### Slug identification

All collected slugs were identified based on morphology and genital morphology and sequences of cytochrome c oxidase subunit I (COI) based on BLAST search results. In total, 20 European populations were investigated, which included 13 populations of *A. vulgaris* (Norway – 9, Poland – 2, France – 1, Germany – 1), and five populations of *A. ater* (all in Norway). Additionally, two populations of hybrids were collected, one population of *A. ater/A. rufus* (Klepp, Norway) and one population of *A. vulgaris/A. rufus* (Zoetermeer, Netherlands). From these sites a total of 648 slugs of the genus *Arion* were collected comprising 490 *A. vulgaris*, 92 *A. ater*, 44 *A. vulgaris/A. rufus* hybrids and 22 *A. ater/A. rufus* hybrids (Table 1).

## Helminth identification and prevalence

Helminths were found associated with slugs at 17 of the 18 sample sites in Europe (94.4%). A total of 501 (77.3%) of 648 examined slugs were infected with helminths. All slug taxa were infected with nematodes. Trematodes were found in *A. vulgaris* and *A. ater*, and cestodes in *A. vulgaris* and *A. ater/A. rufus* hybrids. In *A. vulgaris*, 359 specimens were infected with helminths, i.e., 148 with nematodes (*Alloionema appendiculatum* Schneider, 1859; *Angiostoma* sp.; *Phasmarhabditis hermaphrodita* Schneider, 1859), 198 with trematodes (*Brachylaima mesostoma* Rudolphi, 1803) and 13 with cestodes (*Skrjabinia cesticillus* Molin, 1858 (accession number: AY382321)). In *A. ater*, 74 of the 92 dissected specimens were infected with helminths, i.e., 57 with nematodes (*A. appendiculatum*, *P. hermaphrodita*, *Angiostoma* sp. and *Entomelas* sp.), and 17 with trematodes (*B. mesostoma*, *Eurytrema* sp.). In *A. vulgaris/A. rufus*, 43 of the 44 dissected slugs were infected with nematodes (*A. appendiculatum*). In the 22 dissected *A. ater/A. rufus*, 25 helminths were found, i.e., 23 nematodes (*A. appendiculatum*, *P. hermaphrodita*), and 2 cestodes (*Skrjabinia* sp.) (Table 1).

Nematodes were found in slugs at 14 of the 18 visited sites (Norway – 11, France – 1, Netherlands – 1, Poland – 1; 77.8% of all sample sites), trematodes at 12 of the 18 sites (Norway – 11, France – 1; 66.7% of all sample sites), and cestodes at five of the 18 sites (Norway – 4, Germany – 1; 27.8% of all sample sites). A total of four nematode species (*A. appendiculatum*, *Angiostoma* sp., *P. hermaphrodita*, *Entomelas* sp.), two trematode species (*B. mesostoma*, *Eurytrema* sp.) and one cestode (*Skrjabinia* sp.) species were identified based on morphological and molecular identification (Table 1; explanation regarding the three species *Entomelas* sp., *Eurytrema* sp., and *Skrjabinia* sp. being listed as “genus sp.” is provided in the paragraph “Phylogenetic analysis” below).

From sites positive for nematodes, *A. appendiculatum* was recorded in 11 sites, *P. hermaphrodita* in eight sites, *Angiostoma* sp. in five sites, and *Entomelas* sp. in only one site. For sites positive for trematodes, *B. mesostoma* was found in 11 sites, and *Eurytrema* sp. in only one site. All cestodes were identified as *Skrjabinia* sp. (Table 1).

## Phylogenetic analysis

In total, 501 sequences of the 18S and ITS rDNA regions were generated (Table 1). These sequences represented seven species of helminths from seven families. Sequences of the same species were identical across the 18S and ITS rDNA regions, so only one representative sequence was submitted for each taxon. Obtained sequences of helminth species have been deposited in GenBank with the following accession numbers: KY355082–KY355088 (Table 2).

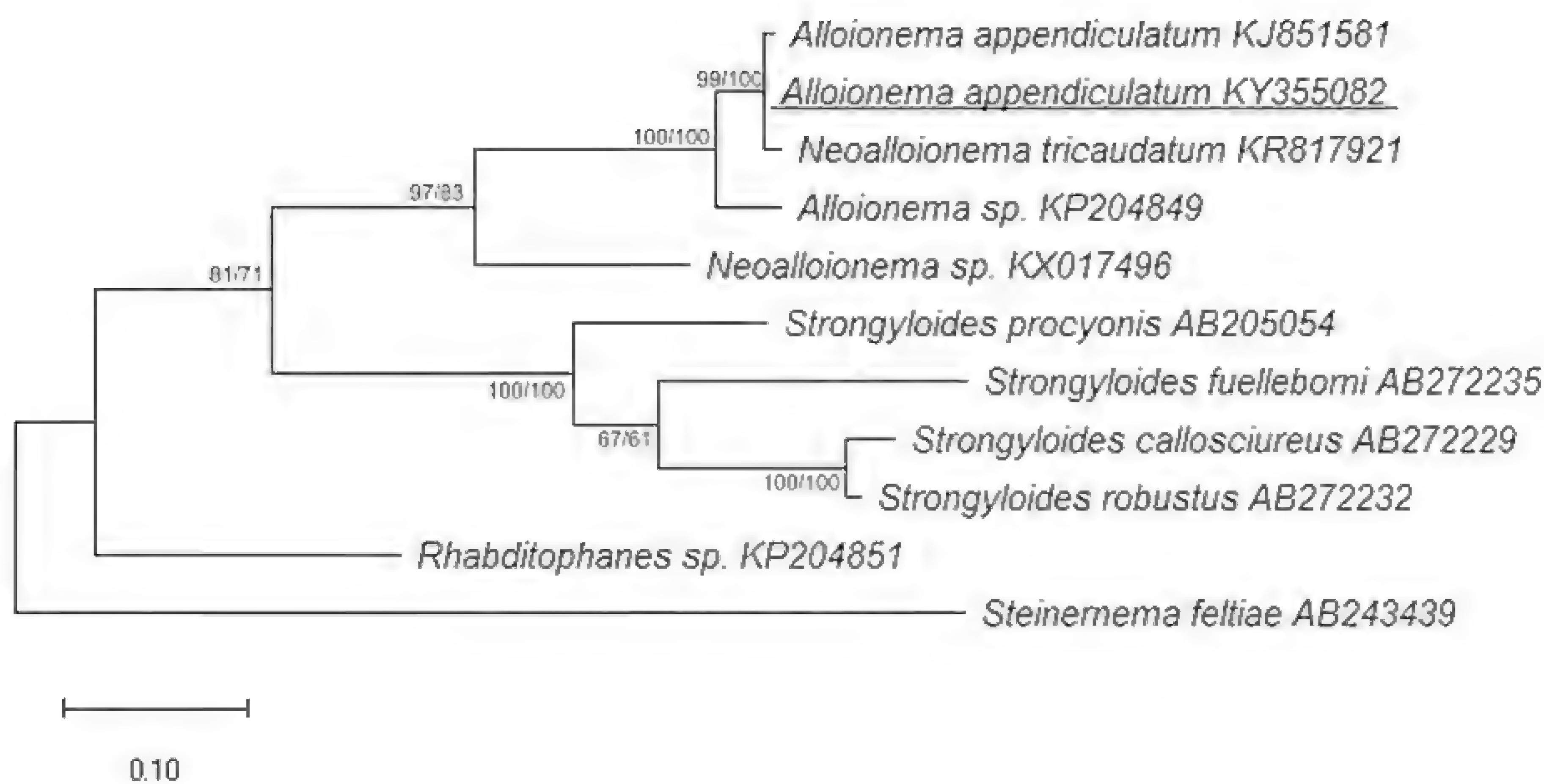
BLAST search for three species sequences did not show exact matches with other sequences deposited in GenBank. The sequence of KY355086 revealed 92.98% identity with *Entomelas dujardini* Maupas, 1916 (accession number: KF999591), sequence of KY355087 revealed 90.46% identity with *Eurytrema pancreaticum* Janson, 1889 (accession number: KY490000), and sequence of KY355088 revealed 83.63% identity with *Skrjabinia cesticillus* Molin, 1858 (accession number: AY382321). Other sequences were characterised by very low query coverage, resulting in low total scores of BLAST searches. None of the sequences available in GenBank were significantly similar to sequences obtained in this study for these three species. Therefore, these three detected helminths (i.e., *Entomelas* sp., *Eurytrema* sp., and *Skrjabinia* sp.) are listed in the study as “genus sp.”

Trees that were inferred from maximum-likelihood (ML) and neighbor joining (NJ) revealed identical topologies. Thus only maximum-likelihood results are presented along with bootstrap support from each method of analysis (Figs 1–6). The molecular phylogenetic trees, generated from partial 18S and ITS of rDNA regions with ML and NJ algorithms, showed that the detected nematodes belonged to the species *A. appendiculatum* (Fig. 1) and *P. hermaphrodita* (Fig. 2), and to the genus *Angiostoma* sp., and *Entomelas* sp. The molecular phylogenetic analysis revealed that these two nematodes are closely related to *Angiostoma margaretae* and *A. norvegicum* (Fig. 3), and *Entomelas dujardini* (Fig. 4). BLAST search for *Angiostoma* sequences revealed 100% identity with *Angiostoma margaretae* Ross, Malan, Ivanova, 2011 (accession number: HQ115062) and *Angiostoma norvegicum* Ross, Haukeland, Hatteland, Ivanova, 2017 (accession number: KU712560). Due to the fact that not enough material was available for molecular work, the 28S and COI could not be used for the identification of these nematodes. Therefore, the nematodes were listed as *Angiostoma* sp. In the study of Singh et al. (2019), partial 18S and D2D3 sequences from the same DNA material were also found to be almost 100% similar to sequences from two different species. The evolutionary history of *A. appendiculatum* was inferred by using the ML method based on the General Time Reversible (GTR) model, and *P. hermaphrodita*, *Angiostoma* sp. and *Entomelas* sp. based on the Tamura 3-parameter model.

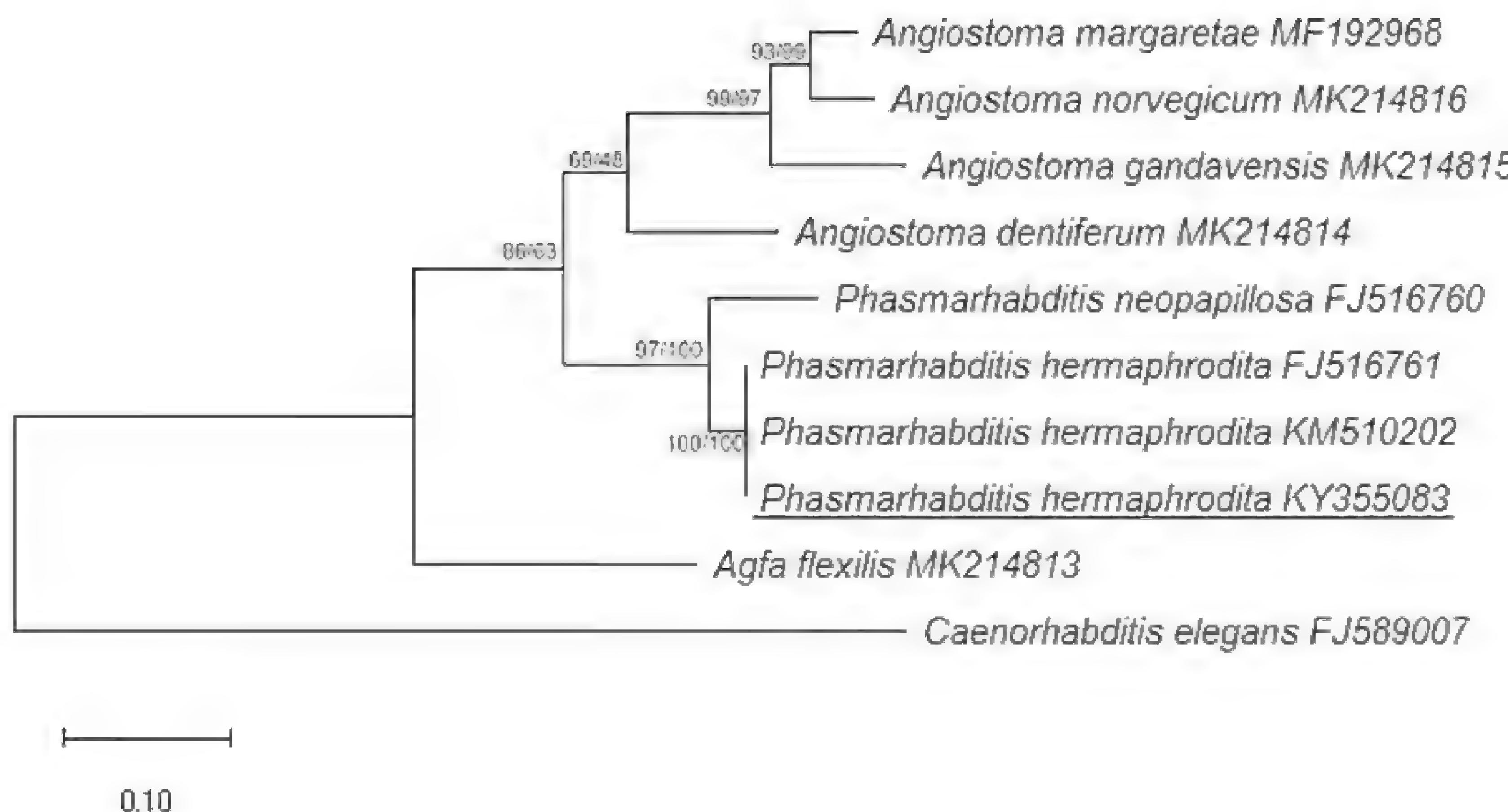
The molecular phylogenetic trees, generated from partial ITS of rDNA regions with ML and NJ algorithms, showed that the detected trematodes belong to the species *B. mesostoma* and to the genus *Eurytrema* sp. (Fig. 5). The molecular phylogenetic analysis revealed that this trematode is closely related to *E. pancreaticum*. The ML method based on the GTR model indicate the evolutionary history of *B. mesostoma* and *Eurytrema* sp. The molecular phylogenetic trees, generated from partial ITS of rDNA regions with ML and NJ algorithms,

**Table 1.** The prevalence (%) of infection of terrestrial slugs with parasite species collected in Europe (N = number of slugs examined).

Locality	GPS coordinates	Slug species	N	Parasite species	%
<b>France:</b>					
Metz	49°7'1" N 6°10'59" E	<i>Arion vulgaris</i>	20	<i>Alloionema appendiculatum</i> <i>Brachylaima mesostoma</i>	65 15
<b>Germany:</b>					
Gauting	48°4'1" N 11°22'1" E	<i>Arion vulgaris</i>	20	<i>Skrjabinia</i> sp.	40
<b>Netherlands:</b>					
Zoetermeer	52°03'54" N 4°30'31" E	<i>Arion vulgaris/</i> <i>A. rufus</i>	44	<i>Alloionema appendiculatum</i>	97.7
<b>Norway:</b>					
Balestrand	61°10'39" N 6°24'14" E	<i>Arion ater</i>	20	<i>Alloionema appendiculatum</i> <i>Brachylaima mesostoma</i> <i>Phasmarhabditis hermaphrodita</i>	45 20 10
Hana, Sandnes	69°31'1" N 20°22'1" E	<i>Arion ater</i>	19	<i>Alloionema appendiculatum</i> <i>Eurytrema</i> sp. <i>Phasmarhabditis hermaphrodita</i>	100 5.3 36.8
Horten	59°25'1" N 10°25'59" E	<i>Arion vulgaris</i>	112	<i>Alloionema appendiculatum</i> <i>Brachylaima mesostoma</i> <i>Phasmarhabditis hermaphrodita</i> <i>Skrjabinia</i> sp.	5.4 87.5 0.9 1.8
Jensvoll, Bodø	67°16'5" N 14°24'0" E	<i>Arion vulgaris</i>	20	<i>Brachylaima mesostoma</i>	25
Kjenneveien, Fredrikstad	59°14'59" N 10°51'30" E	<i>Arion ater</i>	30	<i>Angiostoma</i> sp. <i>Brachylaima mesostoma</i> <i>Entomelas</i> sp.	13.3 40 3.3
Kristiansand	58°10'1" N 8°0'0" E	<i>Arion vulgaris</i>	30	<i>Alloionema appendiculatum</i> <i>Brachylaima mesostoma</i>	6.7 100
Oslo	59°57'41" N 10°53'13" E	<i>Arion vulgaris</i>	25	<i>Angiostoma</i> sp. <i>Phasmarhabditis hermaphrodita</i>	16 16
Lindhjem, Larvik	59°0'42" N 9°58'34" E	<i>Arion vulgaris</i>	39	<i>Alloionema appendiculatum</i> <i>Angiostoma</i> sp. <i>Brachylaima mesostoma</i> <i>Skrjabinia</i> sp.	5.1 7.7 33.3 5.1
Manstad, Fredrikstad	59°16'9" N 10°46'7" E	<i>Arion vulgaris</i>	24	<i>Brachylaima mesostoma</i> <i>Skrjabinia</i> sp.	50 4.2
Klepp	58°46'59" N 5°35'60" E	<i>Arion vulgaris</i>	29	<i>Alloionema appendiculatum</i> <i>Brachylaima mesostoma</i> <i>Phasmarhabditis hermaphrodita</i>	48.3 51.7 3.4
		<i>Arion ater/A. rufus</i>	22	<i>Alloionema appendiculatum</i> <i>Phasmarhabditis hermaphrodita</i> <i>Skrjabinia</i> sp.	95.5 9.1 9.1
Vesterøya, Sandefjord	59°6'14" N 10°14'34" E	<i>Arion ater</i>	13	<i>Angiostoma</i> sp. <i>Phasmarhabditis hermaphrodita</i>	61.5 23.1
Bergen	60°23'37" N 5°22'38" E	<i>Arion ater</i>	10	<i>Alloionema appendiculatum</i> <i>Angiostoma</i> sp. <i>Phasmarhabditis hermaphrodita</i>	10 10 20
		<i>Arion vulgaris</i>	26	<i>Alloionema appendiculatum</i> <i>Angiostoma</i> sp. <i>Brachylaima mesostoma</i> <i>Phasmarhabditis hermaphrodita</i>	38.5 80.8 38.5 50
Time, Bryne	58°43'59" N 5°39'0" E	<i>Arion vulgaris</i>	25	<i>Alloionema appendiculatum</i> <i>Brachylaima mesostoma</i> <i>Phasmarhabditis hermaphrodita</i>	80 48 8
<b>Poland:</b>					
Igołomia	50°5'20" N 20°14'20" E	<i>Arion vulgaris</i>	100	<i>Alloionema appendiculatum</i>	32
Rzeszów	50°1'59" N 22°0'18" E	<i>Arion vulgaris</i>	20		—



**Fig. 1.** Unrooted maximum likelihood phylogeny of ITS rDNA regions for *Alloionema appendiculatum*. The scale bar represents 0.10 substitutions per nucleotide position. Only bootstrap values above 60% are shown.



**Fig. 2.** Unrooted maximum likelihood phylogeny of ITS rDNA regions for *Phasmarhabditis hermaphrodita*. The scale bar represents 0.10 substitutions per nucleotide position. Only bootstrap values above 60% are shown.

showed that the detected cestodes belong to the genus *Skrjabinia* sp. (Fig. 6). The molecular phylogenetic analysis (ML method based on the Hasegawa-Kishino-Yano model) revealed that this cestode is most closely related to *S. cesticillus* and *Raillietina echinobothrida*.

#### Statistical analyses of prevalence

A tendency of higher prevalence of trematodes was found in *A. vulgaris* populations compared with *A. ater* populations (Fig. 7; GLM,  $p=0.0618$ ). On the other hand, a

**Table 2.** The accession numbers of examined slug-parasites with NCBI matches.

Parasite family/species	GenBank no.	NCBI match	Query coverage	Percentage identity	Source
Family: Alloionematidae <i>Alloionema appendiculatum</i>	KY355082	<i>Alloionema appendiculatum</i> ; KJ851581	100	98.95	Nermut' <i>et al.</i> (2015)
Family: Rhabditidae <i>Phasmarhabditis hermaphrodita</i>	KY355083	<i>Phasmarhabditis hermaphrodita</i> ; FJ516761	100	100	—
Family: Angiostomatidae <i>Angiostoma</i> sp.	KY355084	<i>Angiostoma margaretae</i> ; HQ115062 <i>Angiostoma norvegicum</i> ; KU712560	100 100	100 100	Ross <i>et al.</i> (2011) Ross <i>et al.</i> (2017)
Family: Rhabdiasidae <i>Entomelas</i> sp	KY355086	<i>Entomelas dujardini</i> ; KF999591	100	92.98	Tkach <i>et al.</i> (2014)
Family: Brachylaimidae <i>Brachylaima mesostoma</i>	KY355085	<i>Brachylaima mesostoma</i> ; KT074964	100	100	Heneberg <i>et al.</i> (2016)
Family: Dicrocoeliidae <i>Eurytrema</i> sp.	KY355087	<i>Eurytrema pancreaticum</i> ; KY490000	99	90.46	Su <i>et al.</i> (2018)
Family: Davaineidae <i>Skrjabinia</i> sp.	KY355088	<i>Skrjabinia cesticillus</i> ; AY382321	81	83.63	—

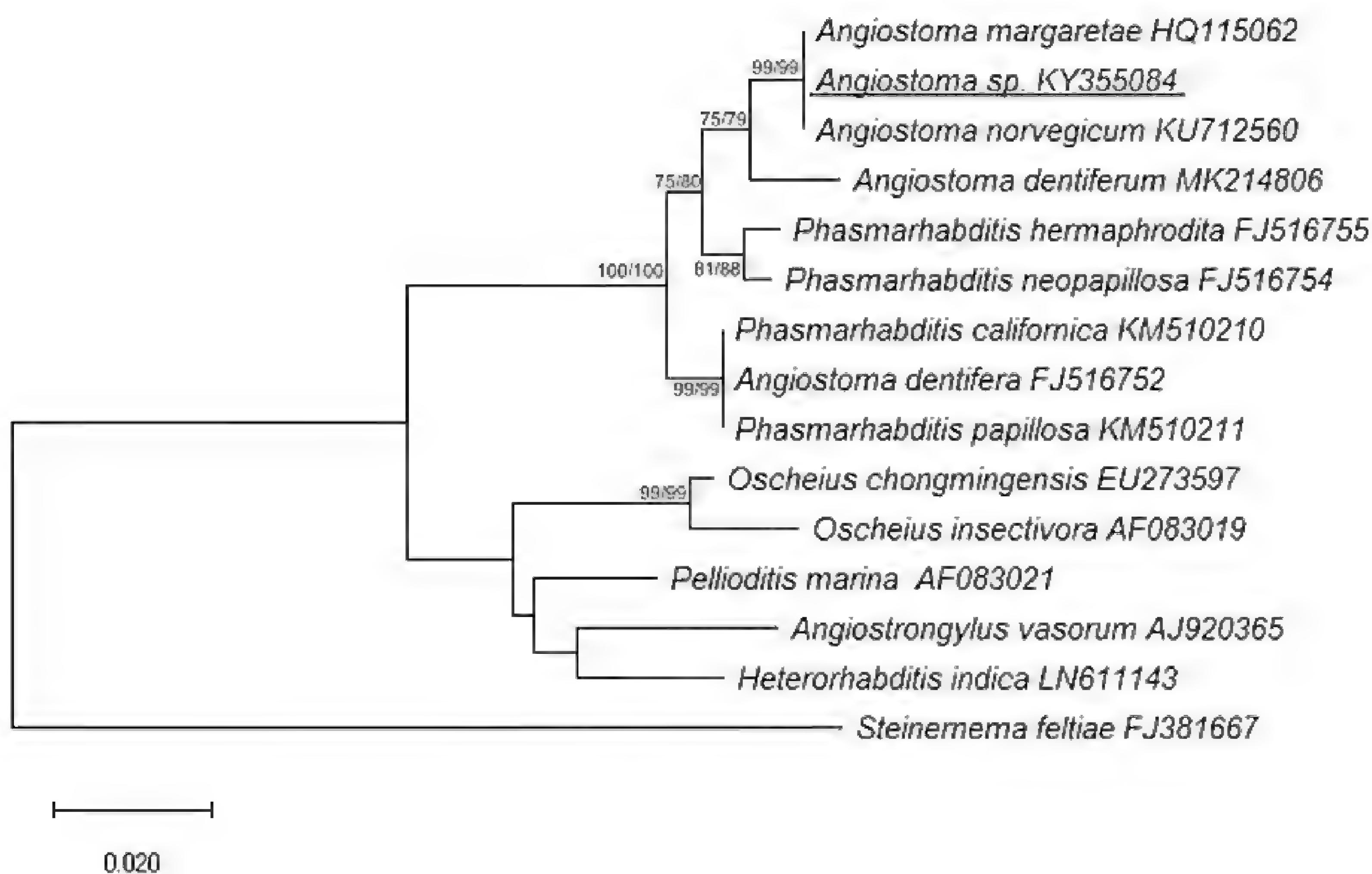
somewhat lower prevalence of nematodes in *A. vulgaris* than in *A. ater* (Fig. 8) was revealed, although this difference was not significant (GLM,  $p=0.1569$ ).

## DISCUSSION

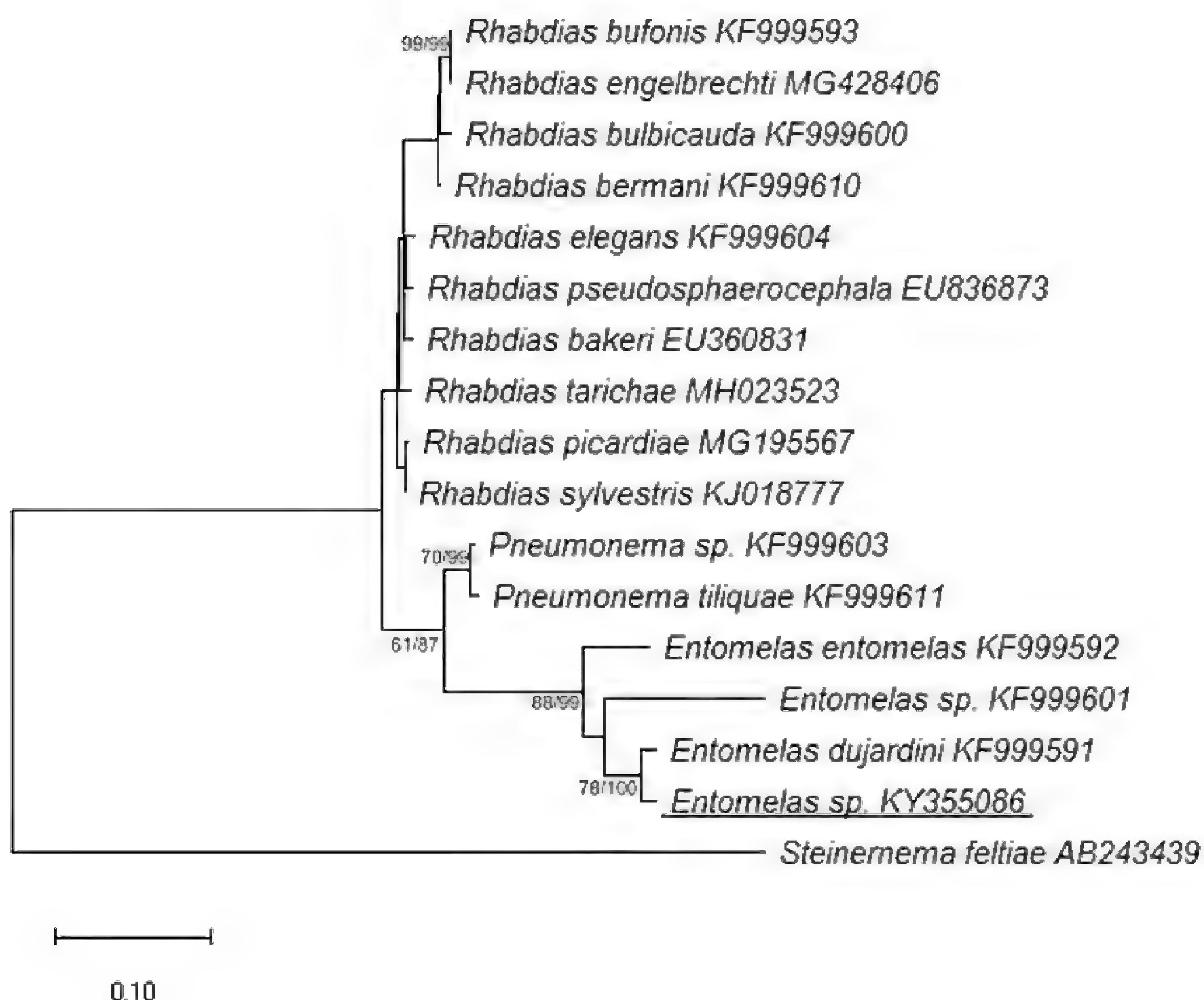
This study presents the occurrence of helminths associated with two slug species: *A. vulgaris* and *A. ater*, as well as *A. vulgaris/A. rufus* hybrids, and *A. ater/A. rufus* hybrids in some parts of Europe, i.e., France, Germany, Netherlands, Norway and Poland. Previous studies on the diversity and distribution of slug parasites have focused on the presence of nematodes in slugs (Mengert 1953; Gleich *et al.* 1977; Charwat & Davies 1999; Laznik *et al.* 2009; Ross *et al.* 2010b; Ross *et al.* 2011; Ivanova *et al.* 2013; Ross *et al.* 2016; Singh *et al.* 2019). In our study, a total of seven species of helminths were found to be associated with these slugs including nematodes, trematodes

and one cestode species. The nematodes were identified to *Alloionema appendiculatum*, *Angiostoma* sp., *Phasmarhabditis hermaphrodita*, *Entomelas* sp., trematodes were identified as *Brachylaima mesostoma*, *Eurytrema* sp., and the cestode species was identified as *Skrjabinia* sp. We found a tendency for higher prevalence of trematodes in *A. vulgaris* compared with the native *A. ater*. However, we did not find the same pattern in terms of prevalence for nematodes.

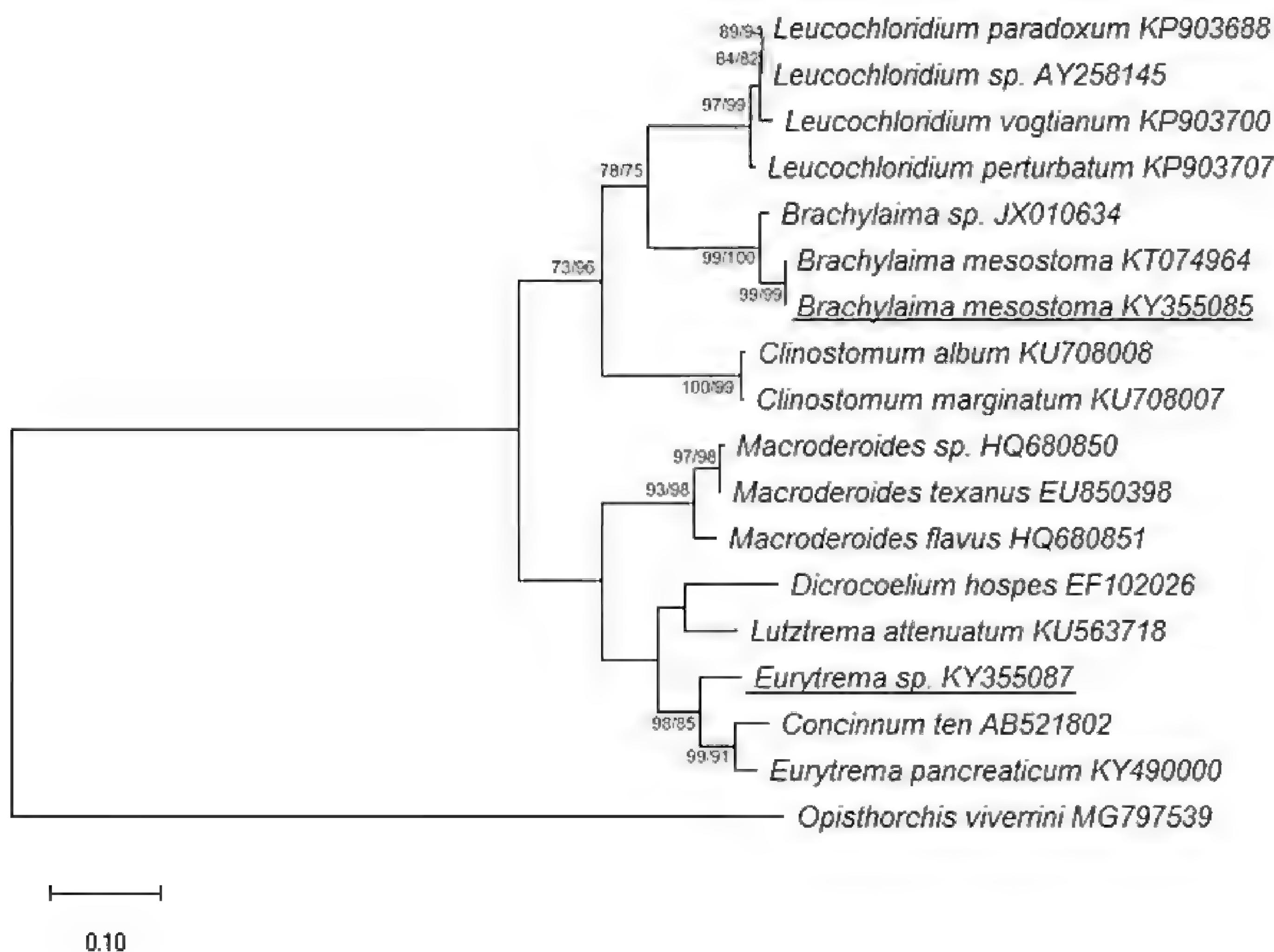
Slug-parasitic nematodes were found in all organs of the body cavity and also on foot muscles, typical of *A. appendiculatum*. The most intensively studied species of slug-parasitic nematode of agricultural and horticultural crops is *P. hermaphrodita*. In our survey, *P. hermaphrodita* was found in eight of the 18 sample sites examined, and was found to parasitize both *A. ater*, *A. vulgaris*, as well as *A. ater/A. rufus* hybrids. Only juveniles of *P. hermaphrodita* were detected in our study. Within the Rhabditidae family, *Phasmarhabditis* is the only genus that



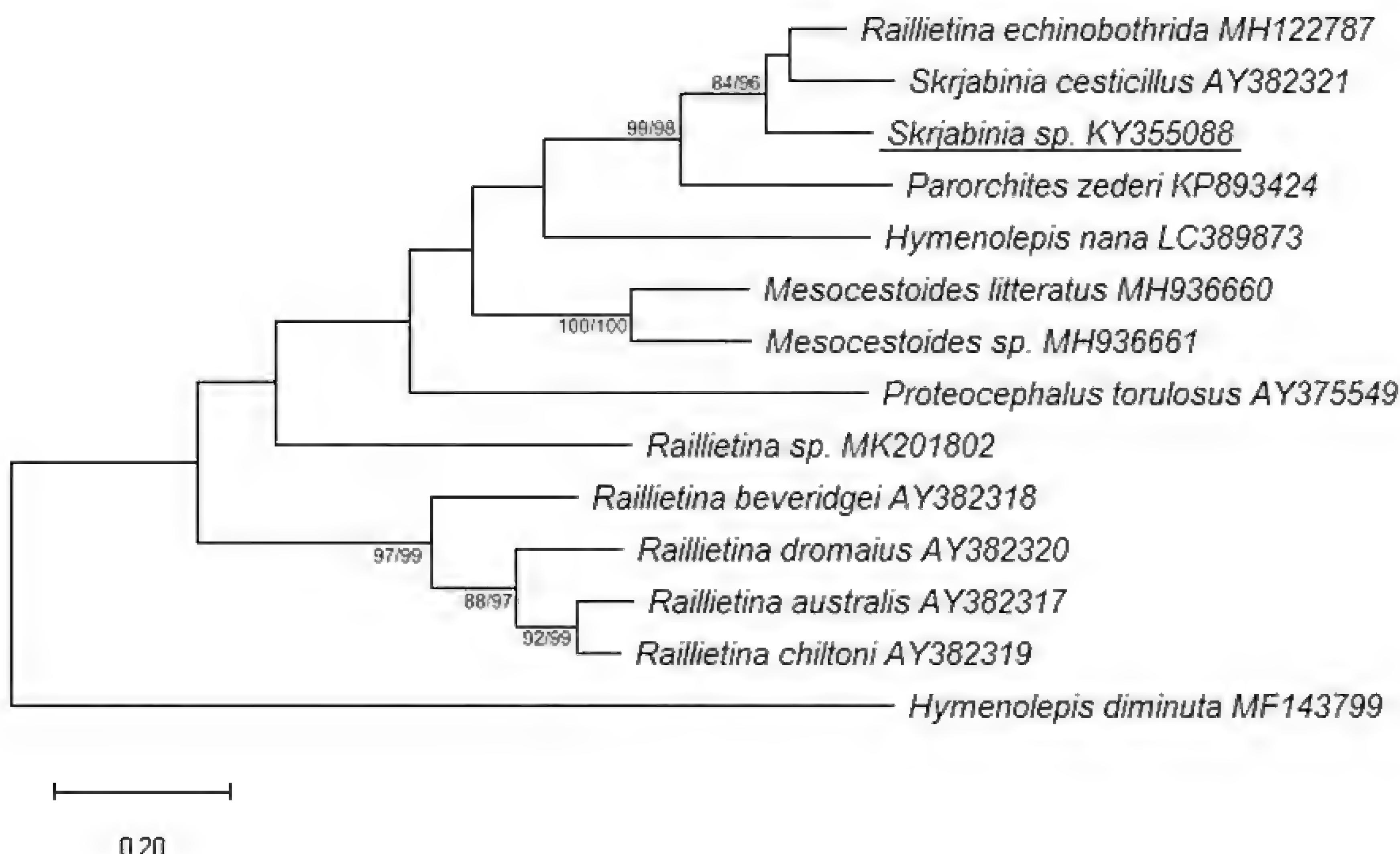
**Fig. 3.** Unrooted maximum likelihood phylogeny of 18S rDNA regions for *Angiostoma* sp. The scale bar represents 0.020 substitutions per nucleotide position. Only bootstrap values above 60% are shown.



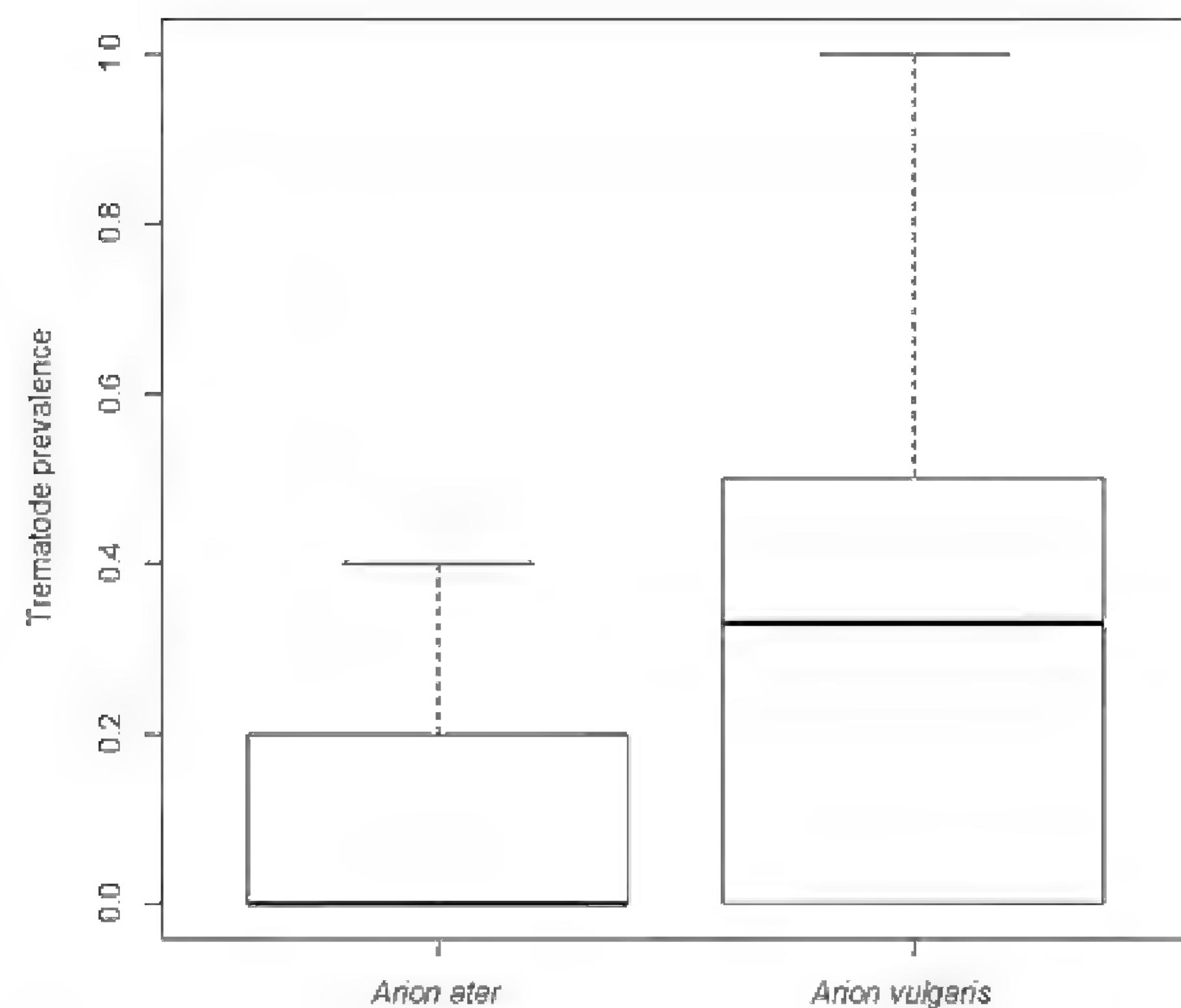
**Fig. 4.** Unrooted maximum likelihood phylogeny of ITS rDNA regions for *Entomelas* sp. The scale bar represents 0.10 substitutions per nucleotide position. Only bootstrap values above 60% are shown.



**Fig. 5.** Unrooted maximum likelihood phylogeny of ITS rDNA regions for *Brachylaima mesostoma* and *Eurytrema* sp. The scale bar represents 0.10 substitutions per nucleotide position. Only bootstrap values above 60% are shown.



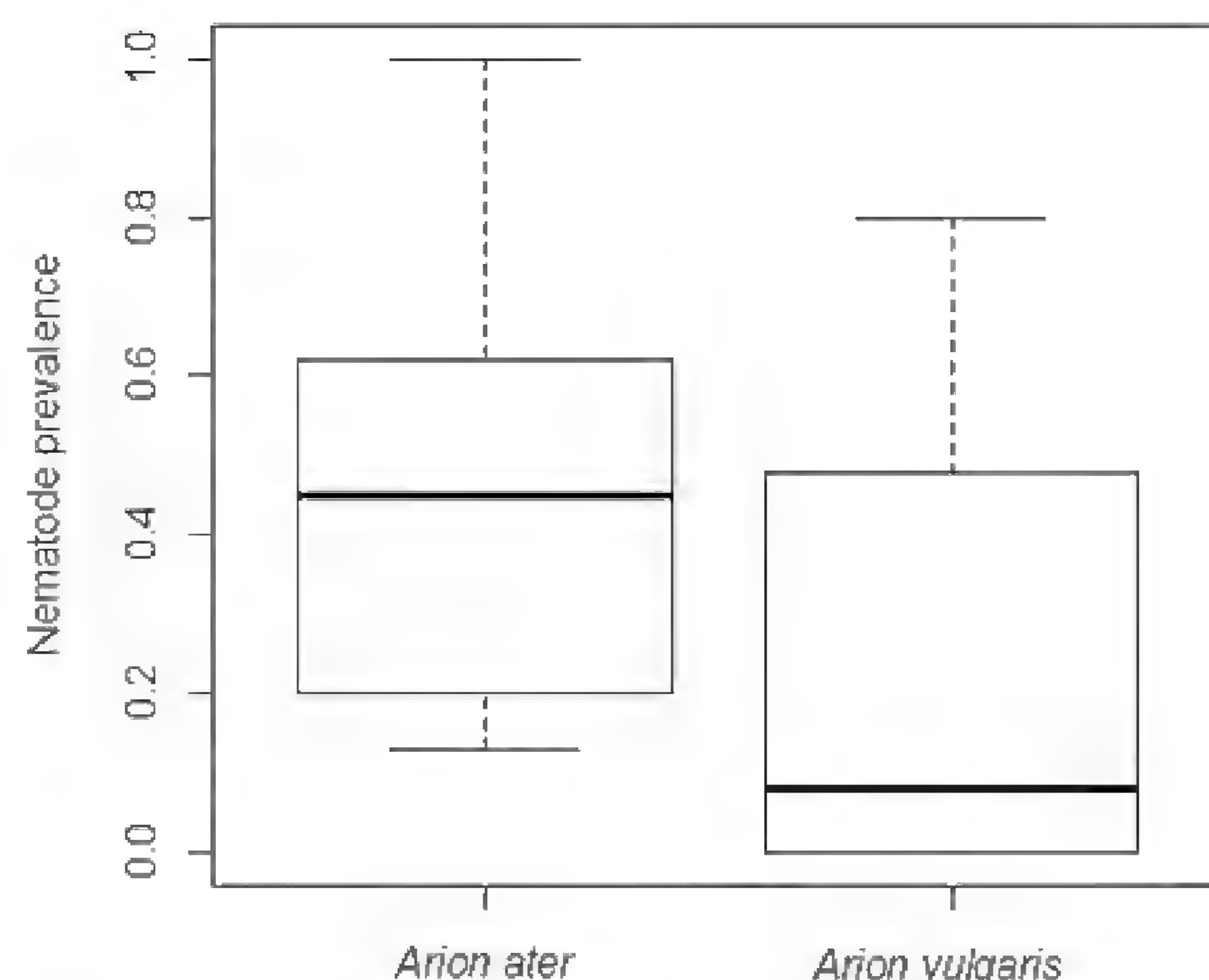
**Fig. 6.** Unrooted maximum likelihood phylogeny of ITS rDNA regions for *Skrjabinia* sp. The scale bar represents 0.20 substitutions per nucleotide position. Only bootstrap values above 60% are shown.



**Fig. 7.** Boxplot of trematode prevalence (proportionally) in populations of *Arion vulgaris* and *A. ater*.

is considered to be truly parasitic towards slugs. *P. hermaphrodita* is known to be capable of killing many species of slugs from several families (Morand et al. 2004; Wilson et al. 2012). The nematode has been formulated into an effective biological control agent (Tan & Grewal 2001; Wilson et al. 2015). Infective dauer juveniles (a non-feeding survival stage) of *P. hermaphrodita* seek out the host through a combination of both chemotactic and chemokinetic responses towards chemical attractants in slug feces and mucus from the foot and mantle (Rae et al. 2006; Hapca et al. 2007).

Another nematode known to be associated with terrestrial gastropods is *Alloionema appendiculatum*. This nematode has a broad geographical distribution and has been found in areas including Europe, Australia and North America (Morand et al. 2004; Laznik et al. 2009; Nermut' et al. 2015). In our study, *A. appendiculatum* was found in 11 of the 18 sample sites examined. The nematode was found to parasitize *A. ater*, *A. vulgaris*, and *A. ater* / *A. rufus* and *A. vulgaris* / *A. rufus* hybrids. *A. appendiculatum* is a common juvenile parasite of many terrestrial gastropods. This nematode has both parasitic and free-living life stages. During the parasitic cycle, third-stage juveniles (J3) enter the slug's body through its foot, where the nematodes moult to the fourth-stage juvenile (J4) which become encapsulated in the pedal musculature. These juveniles then exit the slug and moult into free-living immature adults (Cabaret & Morand 1990; Laznik et al. 2009). In our study we also identified *Angiostoma* sp., present in five of the 18 sample sites examined. The nematode was found to parasitize both *A. ater* and *A. vulgaris*. With the recent discovery of *A. gandavensis*, the total number of described species of the genus



**Fig. 8.** Boxplot of nematode prevalence (proportionally) in populations of *Arion vulgaris* and *A. ater*.

*Angiostoma* is now 19, of which 15 are described from molluscan hosts (Singh et al. 2019). International surveys reveal that molluscan angiostomatids are present in Europe, North America, Africa, South-East Asia and New Zealand (Ivanova & Wilson 2009; Ivanova & Spiridonov 2010; Ross et al. 2010a, b; Ross et al. 2011; Ross et al. 2017).

The other nematode identified in our study was *Entomelas* sp. The nematode was only found in one of the 18 sample sites examined, and only in the native *A. ater*. *Entomelas* sp. is classified in the family Rhabdiasidae, which includes up to 100 known nematode species parasitic in amphibians and reptiles. All share some morphological characters but the most remarkable feature of rhabdiasids is the regular alternation of parasitic and free-living generations (heterogony) in their life cycles (Tkach et al. 2014). *Entomelas dujardini* and *E. entomelas* are commonly associated with *Anguis fragilis*. Experimental infection of the slugs *Deroceras reticulatum* (Agriolimacidae) and *Arion subfuscus* (Arionidae) with infective larvae of *E. entomelas* and *E. dujardini* has revealed that both slug species are classed as paratenic (eu-paratenic) hosts for these nematode species (Kuzmin & Sharpilo 2000).

Among the detected parasites were also trematodes, i.e., *B. mesostoma* and *Eurytrema* sp. We found *B. mesostoma* in 11 of the 18 sample sites examined and it was found in both *A. ater* and *A. vulgaris*. The genus *Brachylaima* contains 72 species that parasitize mammals and birds as definitive hosts around the world, except Antarctica. Terrestrial gastropods are involved as first and second intermediate hosts. They are important from a public health point of view, as they cause diseases

in humans like hemorrhagic enteritis, diarrhea, inflammation of the bile ducts, and anemia (Sirgel et al. 2012; Suleman & Khan 2016; Valente et al. 2016).

The trematode *Eurytrema* sp. was found in one of the 18 sample sites, in *A. ater* only. Species of *Eurytrema* are natural parasites of domestic animals (e.g., cattle, goats, sheep, pigs, dogs) and wild ruminants (such as buffalos, camels, deer) as well as monkeys and humans parasitizing pancreatic ducts and bile ducts. Rarely, terrestrial snails of various species (e.g., *Bradybaena similaris*) are intermediate hosts for these parasites (Pinheiro & Amato 1994). These parasites often cause epithelial hyperplasia, hypertrophy of pancreatic ducts, and periductal fibrosis that lead to eurytrematosis (Cai et al. 2012; Manga-González & Ferreras 2014).

Terrestrial slugs can also be associated with tapeworms. One of the most common tapeworm affecting poultry systems is *Davainea proglottina* and the intermediate hosts are gastropods. Tapeworm segments that pass through poultry feces are ingested by snails and slugs (of the genera *Agriolimax*, *Arion*, *Cepaea* and *Limax*) and within three weeks a cysticercoid is produced. Adult tapeworms are produced in the infected host 8–15 days after ingestion of an infected snail or slug (Jordan & Pattison 1996).

In our study, the tapeworm *Skrjabinia* sp. was found in five of the 18 sample sites examined. The tapeworm was found in *A. vulgaris* and *A. ater/A. rufus* hybrids. *Skrjabinia* is a genus of tapeworms that includes helminth parasites of vertebrates, mostly of birds. One of the most common parasitic platyhelminths in modern poultry facilities throughout the world is *S. cesticillus*. It is a relatively harmless species among intestinal cestodes in spite of a high prevalence. Sometimes called “broad-headed tapeworm”, it infects the small intestine of chicken and occasionally other birds, such as guinea fowl and turkey, which are generally in close proximity to backyard poultry (Kaufmann 1996; Morishita & Schaul 2007). Our study showed that, in some cases, a single slug was infected with up to two different species of parasites (i.e., by two species of nematodes or by one species of nematode and one species of trematode). Two different parasites detected from one host was also sometimes observed in a study by Singh et al. (2019). Moreover, our study confirmed that endoparasitic helminths appear to have a broad host range of slug species (Ross et al. 2010b; Singh et al. 2019). *Alloionema appendiculatum* was reported from all four different slug species, *P. hermaphrodita* from three, *Angiostoma* sp. from two, *B. mesostoma* from two, and *Skrjabinia* sp. also from two.

The enemy release hypothesis suggests that species become invasive due to a lack of enemies in their introduced areas (Mitchell & Power 2003; Torchin et al. 2003). However, Colautti et al. (2004) suggested that there are strong, enemy-specific effects on host survival and that hosts have developed tailored defenses. Thus,

one may expect that it is the release from specific enemies that causes direct changes to survivorship, fecundity, biomass, or demographic variables that really matters. Alternatively, the loss of enemies against which a host is well defended would be of little consequence for the host populations (Colautti et al. 2004). We found a contrasting pattern in our study, suggesting that the prevalence of trematodes in invasive slugs is higher than in native species. The role of trematodes in slugs should be further investigated including comparative studies with nematodes.

The anthropogenic spread of slugs may potentially lead to a higher degree of mixing of slug populations than would occur when spread naturally. This may also include the spread of parasites along with their slug hosts, which may have implications for the prevalence of trematodes and cestodes that use slugs as intermediate hosts and thus may become more common in domestic mammals such as cattle and sheep.

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## APPENDIX

Species name of helminths, GenBank accession numbers and region of sequences used for phylogenetic analyses.

Fig. number	Species name	GenBank accession n°	Region	Source
1	<i>Alloionema appendiculatum</i>	KJ851581	18S-ITS1-5.8S-ITS2-28S	Nermut' <i>et al.</i> (2015)
	<i>Alloionema appendiculatum</i>	KY355082	18S-ITS1-5.8S-ITS2-28S	This study
	<i>Neoalloionema tricaudatum</i>	KR817921	18S-ITS1-5.8S-ITS2-28S	Ivanowa <i>et al.</i> (2016)
	<i>Alloionema</i> sp.	KP204849	18S-ITS1-5.8S-ITS2-28S	Nermut' <i>et al.</i> (2015)
	<i>Neoalloionema</i> sp.	KX017496	18S-ITS1-5.8S-ITS2-28S	Unpublished
	<i>Strongyloides procyonis</i>	AB205054	18S-ITS1-5.8S-ITS2-28S	Sato <i>et al.</i> (2006)
	<i>Strongyloides fuelleborni</i>	AB272235	18S-ITS1-5.8S-ITS2-28S	Sato <i>et al.</i> (2007)
	<i>Strongyloides callosciureus</i>	AB272229	18S-ITS1-5.8S-ITS2-28S	Sato <i>et al.</i> (2007)
	<i>Strongyloides robustus</i>	AB272232	18S-ITS1-5.8S-ITS2-28S	Sato <i>et al.</i> (2007)
	<i>Rhabditophanes</i> sp.	KP204851	18S-ITS1-5.8S-ITS2-28S	Nermut' <i>et al.</i> (2015)
2	<i>Steinernema feltiae</i>	AB243439	18S-ITS1-5.8S-ITS2-28S	Kuwata <i>et al.</i> (2006)
	<i>Angiostoma margaretae</i>	MF192968	18S-ITS1-5.8S-ITS2	Unpublished
	<i>Angiostoma norvegicum</i>	MK214816	18S-ITS1-5.8S-ITS2	Singh <i>et al.</i> (2019)
	<i>Angiostoma gandavensis</i>	MK214815	18S-ITS1-5.8S-ITS2	Singh <i>et al.</i> (2019)
	<i>Angiostoma dentiferum</i>	MK214814	18S-ITS1-5.8S-ITS2	Singh <i>et al.</i> (2019)
	<i>Phasmarhabditis neopapillosa</i>	FJ516760	ITS1-5.8S-ITS2	Unpublished
	<i>Phasmarhabditis hermaphrodita</i>	FJ516761	ITS1-5.8S-ITS2	Unpublished
	<i>Phasmarhabditis hermaphrodita</i>	KM510202	ITS1-5.8S-ITS2	Tandigan <i>et al.</i> (2014)
	<i>Phasmarhabditis hermaphrodita</i>	KY355083	ITS1-5.8S-ITS2	This study
	<i>Agfa flexilis</i>	MK214813	18S-ITS1-5.8S-ITS2	Singh <i>et al.</i> (2019)
3	<i>Caenorhabditis elegans</i>	FJ589007	18S-ITS1-5.8S-ITS2-28S	Imai <i>et al.</i> (2009)
	<i>Angiostoma margaretae</i>	HQ115062	18S	Ross <i>et al.</i> (2011)
	<i>Angiostoma</i> sp.	KY355084	18S	This study
	<i>Angiostoma norvegicum</i>	KU712560	18S	Ross <i>et al.</i> (2017)
	<i>Angiostoma dentiferum</i>	MK214806	18S	Singh <i>et al.</i> (2019)
	<i>Phasmarhabditis hermaphrodita</i>	FJ516755	18S	Ross <i>et al.</i> (2010b)
	<i>Phasmarhabditis neopapillosa</i>	FJ516754	18S	Ross <i>et al.</i> (2010b)
	<i>Phasmarhabditis californica</i>	KM510210	18S	Tandigan <i>et al.</i> (2014)
	<i>Angiostoma dentifera</i>	FJ516752	18S	Ross <i>et al.</i> (2010b)
	<i>Phasmarhabditis papillosa</i>	KM510211	18S	Tandigan <i>et al.</i> (2014)
4	<i>Oscheius chongmingensis</i>	EU273597	18S	Liu <i>et al.</i> (2012)
	<i>Oscheius insectivora</i>	AF083019	18S	Unpublished (2002)
	<i>Pellioiditis marina</i>	AF083021	18S	Unpublished
	<i>Angiostrongylus vasorum</i>	AJ920365	18S	Chilton <i>et al.</i> (2006)
	<i>Heterorhabditis indica</i>	LN611143	18S	Unpublished
	<i>Steinernema feltiae</i>	FJ381667	18S	Unpublished
	<i>Rhabdias bufonis</i>	KF999593	ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2014)
	<i>Rhabdias engelbrechti</i>	MG428406	18S-ITS1-5.8S-ITS2-28S	Kuzmin <i>et al.</i> (2017)

Fig. number	Species name	GenBank accession n°	Region	Source
5	<i>Rhabdias bulbicauda</i>	KF999600	ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2014)
	<i>Rhabdias bermani</i>	KF999610	ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2014)
	<i>Rhabdias elegans</i>	KF999604	ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2014)
	<i>Rhabdias pseudosphaerocephala</i>	EU836873	ITS1-5.8S-ITS2-28S	Dubey & Shine (2008)
	<i>Rhabdias bakeri</i>	EU360831	18S-ITS1-5.8S-ITS2-28S	Dare <i>et al.</i> (2008)
	<i>Rhabdias tarichae</i>	MH023523	18S-ITS1-5.8S-ITS2-28S	Johnson <i>et al.</i> (2018)
	<i>Rhabdias picardiae</i>	MG195567	18S-ITS1-5.8S-ITS2-28S	Svitin <i>et al.</i> (2018)
	<i>Rhabdias sylvestris</i>	KJ018777	ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2014)
	<i>Pneumonema sp.</i>	KF999603	ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2014)
	<i>Pneumonema tiliquae</i>	KF999611	ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2014)
	<i>Entomelias entomelas</i>	KF999592	ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2014)
	<i>Entomelias sp.</i>	KF999601	ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2014)
	<i>Entomelias dujardini</i>	KF999591	ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2014)
	<i>Entomelias sp.</i>	KY355086	ITS1-5.8S-ITS2-28S	This study
	<i>Steinernema feltiae</i>	AB243439	18S-ITS1-5.8S-ITS2-28S	Kuwata <i>et al.</i> (2006)
	<i>Leucochloridium paradoxum</i>	KP903688	ITS1-5.8S-ITS2-28S	Heneberg <i>et al.</i> (2016)
	<i>Leucochloridium sp.</i>	AY258145	ITS1-5.8S-ITS2-28S	Casey <i>et al.</i> (2003)
	<i>Leucochloridium vogtianum</i>	KP903700	ITS1-5.8S-ITS2-28S	Heneberg <i>et al.</i> (2016)
	<i>Leucochloridium perturbatum</i>	KP903707	5.8S-ITS2-28S	Heneberg <i>et al.</i> (2016)
6	<i>Brachylaima sp.</i>	JX010634	5.8S-ITS2-28S	Unpublished
	<i>Brachylaima mesostoma</i>	KT074964	5.8S-ITS2-28S	Heneberg <i>et al.</i> (2016)
	<i>Brachylaima mesostoma</i>	KY355085	5.8S-ITS2-28S	This study
	<i>Clinostomum album</i>	KU708008	18S-ITS1-5.8S-ITS2-28S	Rosser <i>et al.</i> (2017)
	<i>Clinostomum marginatum</i>	KU708007	18S-ITS1-5.8S-ITS2-28S	Rosser <i>et al.</i> (2017)
	<i>Macroderoides sp.</i>	HQ680850	18S-ITS1-5.8S-ITS2-28S	Tkach & Kinsella (2011)
	<i>Macroderoides texanus</i>	EU850398	18S-ITS1-5.8S-ITS2-28S	Tkach <i>et al.</i> (2008)
	<i>Macroderoides flavus</i>	HQ680851	18S-ITS1-5.8S-ITS2-28S	Tkach & Kinsella (2011)
	<i>Dicrocoelium hospes</i>	EF102026	5.8S-ITS2-28S	Maurelli <i>et al.</i> (2007)
	<i>Lutztrema attenuatum</i>	KU563718	5.8S-ITS2-28S	Unpublished
	<i>Eurytrema sp.</i>	KY355087	5.8S-ITS2-28S	This study
	<i>Concinnum ten</i>	AB521802	5.8S-ITS2-28S	Sato <i>et al.</i> (2010)
	<i>Eurytrema pancreaticum</i>	KY490000	18S-ITS1-5.8S-ITS2-28S	Su <i>et al.</i> (2018)
	<i>Opisthorchis viverrini</i>	MG797539	5.8S-ITS2-28S	Sanpool <i>et al.</i> (2018)
	<i>Railletina echinobothrida</i>	MH122787	5.8S-ITS2-28S	Unpublished
	<i>Skrjabinia cesticillus</i>	AY382321	5.8S-ITS2-28S	Unpublished
	<i>Skrjabinia sp.</i>	KY355088	5.8S-ITS2-28S	This study
	<i>Parorchites zederi</i>	KP893424	18S-ITS1-5.8S-ITS2-28S	Kleinertz <i>et al.</i> (2014)
	<i>Hymenolepis nana</i>	LC389873	ITS2	Banzai <i>et al.</i> (2018)
	<i>Mesocestoides litteratus</i>	MH936660	ITS1-5.8S-ITS2-28S	Unpublished
	<i>Mesocestoides sp.</i>	MH936661	ITS1-5.8S-ITS2-28S	Unpublished
	<i>Proteocephalus torulosus</i>	AY375549	5.8S-ITS2-28S	Scholz <i>et al.</i> (2003)

Fig. number	Species name	GenBank accession n°	Region	Source
	<i>Raillietina</i> sp.	MK201802	5.8S-ITS2	Unpublished
	<i>Raillietina beveridgei</i>	AY382318	5.8S-ITS2-28S	Unpublished
	<i>Raillietina dromaius</i>	AY382320	5.8S-ITS2-28S	Unpublished
	<i>Raillietina australis</i>	AY382317	5.8S-ITS2-28S	Unpublished
	<i>Raillietina chiltoni</i>	AY382319	5.8S-ITS2-28S	Unpublished
	<i>Hymenolepis diminuta</i>	MF143799	ITS1-5.8S-ITS2	Unpublished

## Research article

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### The Ladybird Beetles (Coleoptera: Coccinellidae) of Arunachal Pradesh, East Himalaya, India with new combinations and new country records

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**Abstract.** The present communication on ladybird beetles (Coccinellidae) of Arunachal Pradesh (AP), India, a part of Eastern Himalayan biodiversity hotspot, reports a total of 44 species belonging to 22 genera and 6 tribes. Thirty-eight species were examined and illustrated, including three new species records from India: *Illeis shensiensis* Timberlake, 1943, *Afissa rana* (Kapur, 1958), and *Henosepilachna vigintioctomaculata* (Motschulsky, 1857) (first confirmed record from India), and 26 species as new to Arunachal Pradesh. *Epilachna gibbera* Crotch, 1874, *E. mystica* Mulsant, 1850, and *E. undecimspilota* Hope, 1831 are transferred to the genus *Afissa*.

**Key words.** Checklist, new distributional records, taxonomy, Himalaya.

## INTRODUCTION

The family Coccinellidae include around 6,000 described species belonging to 360 genera and 25 tribes under superfamily Coccinelloidea globally (Seago et al. 2011; Robertson et al. 2015), of which approximately 430 species are known from India (Jadwiszczak & Wegrzynowicz 2003; Poorani 2002b, 2004). The coccinellid fauna of Indian part of Himalaya is represented by more than 203 species with most of them recorded from North-west (68 species), Western (107 species), and Central Himalaya (133 species), and the eastern Himalaya in the state of Arunachal Pradesh (AP) remains largely unexplored (Gupta et al. 2018). The recent contributions on ladybird beetle fauna of AP have been made by Poorani & Booth (2016), Poorani & Sambath (2017), Poorani & Thangjam (2019), and Poorani (2019). Therefore, the present study is intended to fill this gap in the distribution and report the previously unrecorded species from the state, along with the new additions to the ladybird beetle fauna of India.

The present study, collectively with the previously published data (6 species), reports a total of 44 species of ladybird beetles belonging to 22 genera and 6 tribes of family Coccinellidae from the state. Among them, 38 species were examined and illustrated (Figs 1–53). Whereas, *Renius cornutus* Li & Wang, 2017, *Halyzia nepalensis* Canepari, 2003, *Halyzia sanscrita* Mulsant, 1853, *Harmonia manillana* (Mulsant, 1866), *Micraspis unicus* Poorani, 2019, and *Oenopia chinensis* (Weise, 1912) were included from literature. All the species are

listed with their valid names along with their major citations and their distribution in AP (in districts), India (in states) and outside India. *Illeis shensiensis* Timberlake, 1943 and *Afissa rana* (Kapur, 1958) are recorded for the first time from India along with the first verified record of *Henosepilachna vigintioctomaculata* (Motschulsky, 1857) from India, which was listed from India by Poorani (2004) in her updated checklist, but without any specific locality data. Twenty-six species have been reported for the first time from the state. New combinations are proposed here for three species of Epilachini: *Afissa gibbera* (Crotch, 1874), comb. nov., *Afissa mystica* (Mulsant, 1850), comb. nov., and *Afissa undecimspilota* (Hope, 1831), comb. nov., based on the phylogenetic classification and revision of the world genera of tribe Epilachnini by Tomaszewska & Szawaryn (2016). These species were previously included in the genus *Epilachna*.

## MATERIALS AND METHODS

The materials for the present study collected during recent faunistic surveys to Dihang-Dibang Biosphere Reserve, Namdapha National Park, and Tawang districts of AP along with the specimens, deposited at the Coleoptera Section of Zoological Survey of India, Kolkata. The specimens were identified with the help of following publications: Dieke (1947), Kapur (1946, 1948), Bielawski (1961), Iablokoff-Khnzorian (1982), Booth (1997), Poorani & Booth (2006), Poorani et al. (2008),

Ren et al. (2009), and Tomaszewska & Szawaryn (2016). The specimen identifications were verified also with Dr A.P. Kapur's Coccinellidae collection present at the Coleoptera Section, ZSI. Wherever required, the male genitalia was also dissected, cleaned in 10% KOH solution, and studied for confirming the identity of the species. The specimens were examined using a Nikon SMZ25 stereo zoom-microscope, and the photographs were taken using DS-Ri2 camera with NIS Elements BR 5.10.00 imaging software. Images were also slightly modified using Adobe Photoshop CS3. Scanning electron microscopy technique was also used and the images were taken using Carl Zeiss EVO18. The species with a single asterisk mark (\*) are newly recorded from AP whereas with two asterisks marks (\*\*) are recorded for the first time from India. The material examined in each species broadly includes the district in AP, micro locality, date of collection, number of examples, and collector name. The specimens are deposited in the National Zoological Collection of Zoological Survey of India, Kolkata (NZSI).

### Institutional abbreviations

NZSI = Zoological Survey of India, M Block, New Alipore, Kolkata, 700053, India;  
 ZSI-CZRC = Zoological Survey of India, Central Zone Regional Centre, Vijay Nagar, Jabalpur, 482002, Madhya Pradesh;  
 NBAIR = National Bureau of Agricultural Insect Resources, Bellary Road, Bengaluru, 560024, Karnataka, India;  
 SCAU = South China Agriculture University, Guangzhou, 510640, China.

## RESULTS

### Taxonomic Account

#### Family Coccinellidae Latreille, 1807

#### Subfamily Coccinellinae Latreille, 1807

#### Tribe Aspidimerini Mulsant, 1850

#### Genus *Cryptogonus* Mulsant, 1850

**1. *Cryptogonus bimaculatus* Kapur, 1948** (Fig. 1)  
*Cryptogonus bimaculatus* Kapur, 1948: 100, fig. 8D.

**Material examined.** Papum Pare: Banderdewa, 11.iv.2001 (1 ♂), leg. Sheela.

**Distribution.** India: Arunachal Pradesh (Papum Pare), Assam, Nagaland, and Tamil Nadu. Elsewhere: Bhutan, China, Myanmar, Nepal, and Thailand (Kapur 1948; Canepari 1997; Poorani 2002b, 2004; Kovář 2007; Ren et al. 2009).

#### **2. *Cryptogonus quadriguttatus* (Weise, 1895) (Fig. 2)\***

*Aspidiphorus quadriguttatus* Weise, 1895: 326.

*Cryptogonus quadriguttatus*: Weise, 1900: 428; Kapur, 1948: 97, fig. 7A–I.

*Cryptogonus quadriguttatus* var. *confluens* Kapur, 1948: 99.

*Cryptogonus quadriguttatus* var. *nigriscens* Kapur, 1948: 99.

**Material examined.** Papum Pare: Banderdewa, 11.iv.2001 (1 ♀) (25402/H4A), leg. Sheela.

**Distribution.** India: Arunachal Pradesh (Papum Pare), Assam, Goa, Nagaland, Uttar Pradesh, Uttarakhand, Sikkim, Tripura, and West Bengal. Elsewhere: Bhutan, and China (Kapur 1948, 1963; Poorani 2002b, 2004).

#### Tribe Chilocorini Mulsant, 1846

#### Genus *Renius* Li & Wang, 2017

#### **3. *Renius cornutus* Li & Wang, 2017**

*Renius cornutus* Li & Wang, 2017 in Li et al. 2017: 122, 124.

**Distribution.** India: Arunachal Pradesh (Tiwariagaon). Elsewhere: China (Poorani & Thangjam, 2019).

**Remarks.** *R. cornutus* was described by Li & Wang (2017) from China (Tibet), the type material of which is deposited at SCAU. Recently, Poorani & Thangjam (2019) identified and reported this species from Arunachal Pradesh, based on a female specimen, deposited at NBAIR. We lack this species in our collection, therefore could not examine the material of the species.

#### Genus *Priscibrumus* Kovar, 1997

#### **4. *Priscibrumus uropygialis* Mulsant, 1853 (Fig. 3)\***

*Exochomus uropygialis* Mulsant, 1853: 196.

*Brumus uropygialis*: Crotch, 1874: 196.

*Exochomus (Exochomus) uropygialis*: Barovsky, 1922: 297; Miyatake, 1985: 11–12, figs 19–22.

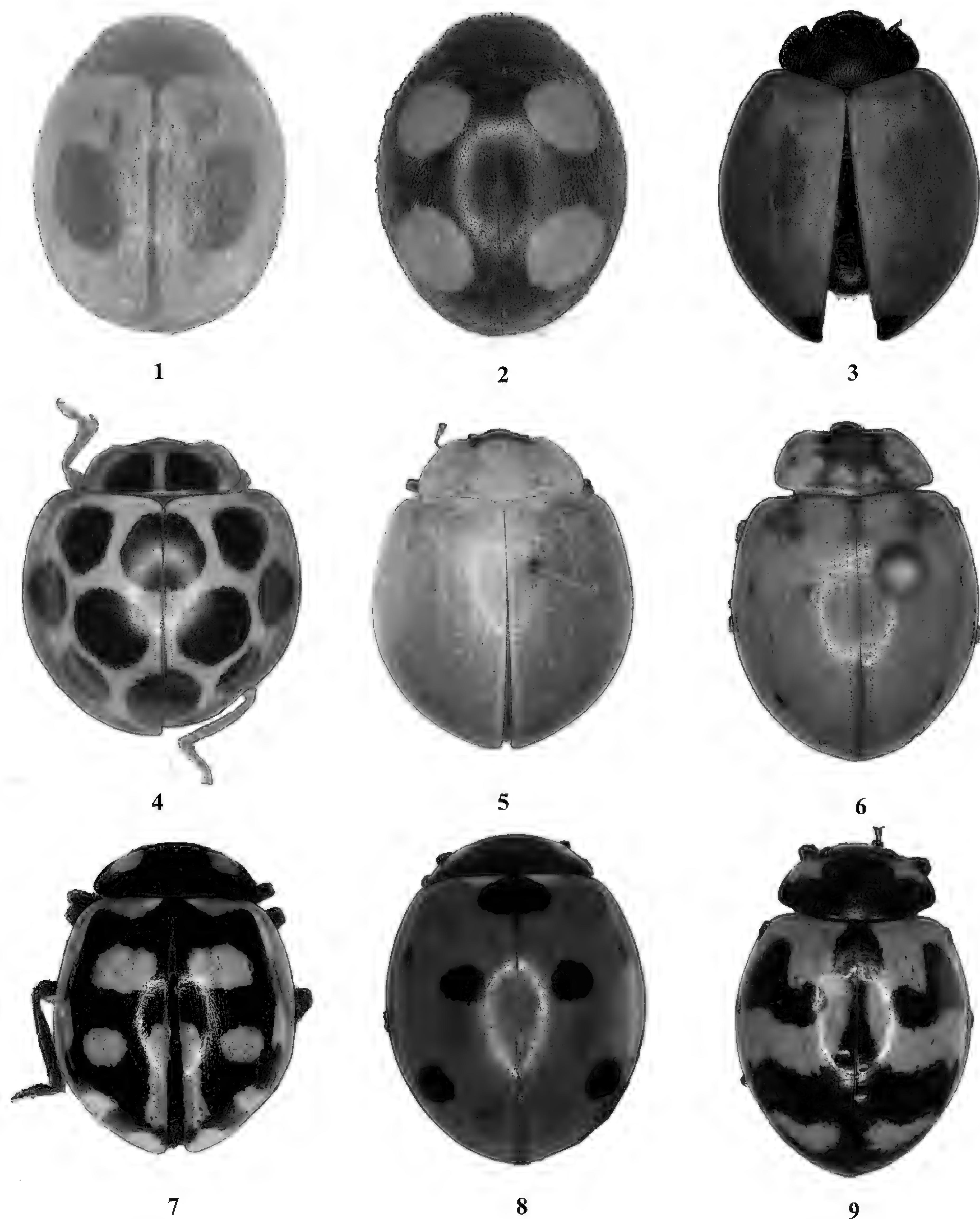
*Priscibrumus uropygialis*: Kovář, 1997: 117.

**Material examined.** Changlang: Namdapha National Park, 24.vi.2017 (1ex.) (24904/H4A), 361m, leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Changlang), Himachal Pradesh, and Jammu & Kashmir. Elsewhere: Bhutan, Nepal, and Pakistan (Bielawski 1979; Canepari 1997; Poorani 2002b, 2004).

#### Tribe Coccinellini Latreille, 1807

#### Genus *Alloneda* Iablokoff-Khnzorian, 1979



**Figs 1–9.** Habitus of (1) *Cryptogonus bimaculatus* Kapur, 1948; (2) *Cryptogonus quadriguttatus* (Weise, 1895); (3) *Priscibrumus uropygialis* Mulsant, 1853; (4) *Alloneda dodecaspilota* (Hope, 1831); (5) *Calvia albida* Bielawski, 1972; (6) *Calvia sykesii* (Crotch, 1874); (7) *Coccinella luteopicta* (Mulsant, 1866); (8) *Coccinella septempunctata* Linnaeus, 1758; (9) *Coccinella transversalis* Fabricius, 1781.

**5. *Alloneda dodecaspilota* (Hope, 1831) (Fig. 4)\****Coccinella 12-spilota* Hope, 1831: 31.*Caria duodecimspilota*: Mulsant, 1850: 236.*Aiolocaria dodecaspilota*: Crotch, 1874: 178; Kapur, 1963, 26, fig. 9A.*Palaeoneda dodecaspilota*: Mader, 1934: 302.*Alloneda dodecaspilota*: Iablokoff-Khnzorian, 1982: 277, fig. 45b; Miyatake, 1985: 20, figs 38–42.**Material examined.** West Kameng: Bokhar, 27.v.1961 (2 exs) (25302/H4A), 2800m, leg. K.C. Jayram.**Distribution.** India: Arunachal Pradesh (West Kameng), Himachal Pradesh, Sikkim, and West Bengal. Elsewhere: Bhutan, China, Myanmar, Nepal, Thailand, and Vietnam (Miyatake 1985; Canepari 1997; Poorani 2002b, 2004).**Genus *Calvia* Mulsant, 1846****6. *Calvia albida* Bielawski, 1972 (Fig. 5)\****Calvia albida* Bielawski, 1972: 308, figs 131, 132–139; Booth, 1997: 931, fig. 28, 41; Poorani, 2014: 4, figs 1d, 3.**Material examined.** Tawang: Jang, 25.ix.2013 (2 exs) (25398/H4A), leg. P.P.B.**Distribution.** India: Arunachal Pradesh (Tawang), Manipur, Sikkim, Uttar Pradesh, and West Bengal. Elsewhere: Nepal (Poorani 2002b, 2004; Poorani & Sambath, 2017).**7. *Calvia sykesii* (Crotch, 1874) (Fig. 6)\****Anisocalvia sykesii* Crotch, 1874: 146.*Calvia sykesii*: Korschefsky, 1932: 529; Booth, 1997: 930, fig. 27.**Material examined.** Changlang: Namdapha National Park, Deban, 361m, 24.vi.2017 (4 exs) (24906/H4A), leg. J. Saini.**Distribution.** India: Arunachal Pradesh (Changlang), Assam, Meghalaya, Sikkim, Tamil Nadu, and West Bengal. Elsewhere: Nepal (Booth 1997; Poorani 2002b, 2004).**Genus *Coccinella* Linnaeus, 1758****8. *Coccinella luteopicta* (Mulsant, 1866) (Fig. 7)\****Adalia luteopicta* Mulsant, 1866: 45.*Lioadalia luteopicta*: Crotch, 1874: 104; Bielawski, 1971: 7–8, figs 27–35.*Coccinella luteopicta*: Iablokoff-Khnzorian, 1982: 395; Canepari, 1997: 52.**Material examined.** East Kameng: Seppo, 3500m, 11.x.1996, (1 ex.) (25303/H4A), leg. S.K. Mondal. Tawang: 24.ix.2013, (1 ex.) (25304/H4A), leg. P.P.B; Jang: 26.ix.2013 (1 ex.) (25305/H4A), leg. J. Majumder.**Distribution.** India: Arunachal Pradesh (East Kameng, Tawang), Himachal Pradesh, Sikkim, Uttar Pradesh, and West Bengal. Elsewhere: Bhutan, China, and Nepal (Poorani 2002b, 2004; Kovář 2007).**9. *Coccinella septempunctata* Linnaeus, 1758 (Fig. 8)***Coccinella 7-punctata* Linnaeus, 1758: 365.*Coccinella septempunctata*: Korschefsky, 1932: 486.*Coccinella divaricata* Olivier, 1808: 1001.*Coccinella confusa* Wiedemann, 1823: 72.*Coccinella bruckii* Mulsant, 1866: 90; Crotch, 1874: 46.*Coccinella septempunctata bruckii*: Korschefsky, 1932: 491.**Material examined.** West Kameng: Kalaktang, Stn. 17, 17.iii.1961, (1 ex.), Rahang, Stn. 34, 17.iv.1961, (1 ex.), Bomdila Pass, Stn. 33, 17.iv.1961, (1 ex.), Shergaon, Stn. 51, 05.v.1961, (1 ex.), Shergaon, Stn. 25, 08.v.1961, (2 exs), Moshing, Stn. 22, 11.v.1961, (2 exs), But Vill. Stn. 61, 24.vi.1961, (1 ex.), leg. K.C. Jayram. Gandhigram: 22.ii.1988 (1 ex.), 26.ii.1988 (1 ex.). Papum Pare: Kokila North of Chessa, 21.x.1996 (1ex.) (24302/H4A), leg. A.M. Biswas. Roing: Debang Valley, 25.i.2000 (1 ex.) (24303/H4A), Deopani, 250m, 29.i.2000 (2 exs) (25306/H4A), leg. S.K. Mondal. Ziro: Joram, 2.vi.2000 (3 exs) (24304/H4A), leg. B. Mitra. West Kameng: Tenga, 24.iv.2001 (1 ex.) (25307/H4A), leg. S. Sheela. Changlang: Namdapha National Park, Lankhal Nala, 6.iii.2017 (1 ex.) (25308/H4A), Deban River Bed, 345m, 12.iii.2017 (2 exs) (24309/H4A), Anamika Fall, 413m, 13.iii.2017 (1 ex.) (25310/H4A), leg. J. Saini. Tawang: Zemithang, 24.vi.2017 (3 exs) (24911/H4A), Lumla, 24.vi.2017 (1 ex.) (25311/H4A), 11.iv.2018 (1 ex.) (24912/H4A), Namtsering, 15.iv.2018 (1 ex.) (24913/H4A)/16.iv.2018 (1 ex.) (24914/H4A), 17.iv.2018 (7 exs) (24915/H4A), leg. J. Saini. Dihang Dibang Biosphere Reserve, Maliny, 25.x.2017 (5 exs) (24437/H4A), leg. D. Gupta.**Distribution.** Widely distributed throughout India including Arunachal Pradesh (Gandhigram, Papum Pare, Roing, Ziro, West Kameng, Changlang, Tawang, Dihang Dibang Biosphere Reserve). Elsewhere: China, Afro-tropical Region, North America, Pakistan, and Sri Lanka (Poorani 2002b, 2004; Kovář 2007; Poorani & Sambath 2017).**10. *Coccinella transversalis* Fabricius, 1781 (Fig. 9)\****Coccinella transversalis* Fabricius, 1781: 97; Iablokoff-Khnzorian, 1979: 68.*Coccinella repanda* Thunberg, 1781: 18.**Material examined.** Papum Pare: Kokila North of Chessa, 21.x.1996 (1ex.) (24299/H4A), leg. A.M. Biswas; Balijan, 23.x.1996 (1ex.) (24300/H4A), leg. S.K. Mondal; Baderdewa: 11.iv.2001 (2 exs) (24301/H4A), leg. Sheela. East Siang: Boleng, 18.i.2000 (1 ex.) (25312/

H4A), leg. S.K. Mondal. Dihang Dibang Biosphere Reserve, Maliny, 25.x.2017 (4 exs) (24436/H4A), leg. D. Gupta.

**Distribution.** Widely distributed in throughout India including Arunachal Pradesh (Papum Pare, Baderdewa, East Siang, Dihang Dibang Biosphere Reserve). Elsewhere: Australia, Bangladesh, Indochina, Indonesia, Japan, Nepal, New Zealand, and Sri Lanka (Poorani 2002b, 2004).

**Remarks.** It is commonly distributed species in India but was not earlier reported from the state of Arunachal Pradesh.

#### Genus *Coelophora* Mulsant, 1850

##### 11. *Coelophora bissellata* Mulsant, 1850 (Fig. 10)\*

*Coelophora bissellata* Mulsant, 1850: 400.

*Spilocaria bissellata*: Timberlake, 1943: 58.

*Lemnia (Spilocaria) bissellata*: Iablokoff-Khnzorian, 1979: 62.

*Lemnia bissellata*: Hoang, 1983: 74; Iablokoff-Khnzorian, 1982: 218.

*Caria gracilicornis* Weise, 1902: 505.

**Material examined.** Siang, NEFA: 10.xi.1971 (1 ex. on leaves,) (25318/H4A), leg. S. Ghose. Papum Pare: Kokila, North of Chessa, 21.x.1996 (1 ex.) (25315/H4A), leg. A.M. Biswas; Banderdewa, 11.iv.2001 (1 ex.) (25320/H4A), leg. Sheela. Debang Valley: Kannu North, 1.ii.2000 (1 ex.) (25322/H4A), leg. S.K. Mondal. Roing: Dibang Valley, 29.ix.2000 (1 ex.) (25319/H4A), leg. R.S. Mridha. Itanagar: Ganga Lake, 28.v.2000 (4 exs) (25314/H4A), leg. T.K. Mondal; Naharlagun, 530m, 29.v.2000 (1 ex.) (25321/H4A), leg. K. Bhattacharya. West Siang: Aalo (formerly Along), Hissan Colony, 11.vi.2000 (2 exs) (25317/H4A), leg. R.S. Mridha. East Kameng: Rang, Gthili, 17.ix.2000 (5 exs) (25313/H4A), leg. A.R. Lahiri. Changlang, Deban Rest House, 4.iii.2017 (1 ex.) (25316/H4A), leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Siang, Papum Pare, Roing, Debang Valley, West Siang, East Kameng, Changlang), Assam, Himachal Pradesh, Karnataka, Kerala, Manipur, Meghalaya, Sikkim, Tamil Nadu, Uttar Pradesh, and West Bengal. Elsewhere: Bhutan, Bangladesh, China, Indonesia, Nepal, New Guinea, Philippines, Thailand, and Vietnam (Poorani 2002b, 2004; Kovář 2007).

#### Genus *Halyzia* Mulsant, 1846

##### 12. *Halyzia dejavu* Poorani & Booth, 2006 (Fig. 11)

*Halyzia dejavu* Poorani & Booth, 2006: 66, pl. 1B, figs 10–17.

**Material examined.** Changlang: Deban, 24.xii.2017 (1 ex.) (24905/H4A), leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Changlang, Tawang), Sikkim; Nepal (Poorani & Booth 2006; Poorani & Sambath 2017).

#### 13. *Halyzia nepalensis* Canepari, 2003

*Halyzia nepalensis* Canepari 2003: 261, figs 1–2.

**Distribution.** India: Arunachal Pradesh. Elsewhere: Myanmar, and Nepal (Poorani & Thangjam 2019).

**Remarks.** Poorani & Thangjam (2019) reported this species from Arunachal Pradesh, based on photographic records, which they found identical with the specimens from Myanmar, deposited at NZSI. The specimens of this species from Arunachal Pradesh were unavailable with us for examination.

##### 14. *Halyzia sanscrita* Mulsant, 1853

*Halyzia sanscrita* Mulsant, 1853: 152.

**Distribution.** India: Arunachal Pradesh (Tawang), Himachal Pradesh, Sikkim, and Uttarakhand. Elsewhere: Bhutan, China, and Nepal (Poorani 2002b, 2004; Kovář 2007; Poorani & Sambath 2017).

**Remarks.** Poorani & Sambath (2017) recorded this species from Tawang, Arunachal Pradesh, based on the specimens, deposited at ZSI-CZRC, which were examined by the authors.

#### Genus *Harmonia* Mulsant, 1846

##### 15. *Harmonia dimidiata* (Fabricius, 1781) (Fig. 12)

*Coccinella dimidiata* Fabricius, 1781: 94.

*Coccinella dimidia* Hope, 1831: 30.

*Leis dimidiata*: Mulsant, 1850: 242.

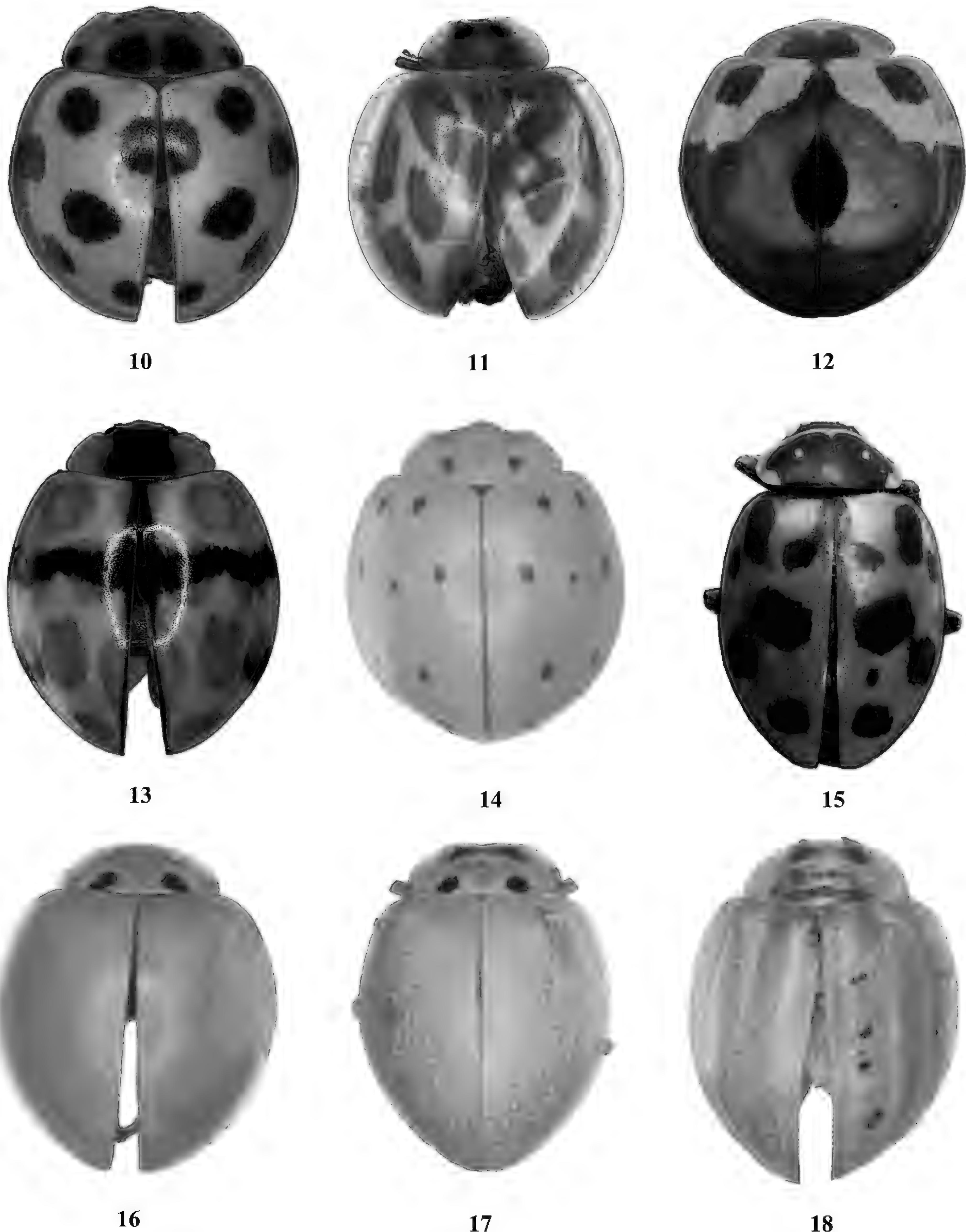
*Coccinella quindecimmaculata* Hope, 1831: 30.

*Coccinella bicolor* Hope, 1831: 31.

*Harmonia dimidiata*: Miyatake, 1965: 62.

**Material examined.** West Kameng: Salari, Stn. 60, 22.vi.1961, (2 exs), But village, Stn. 61, 24.vi.1961, (1 ex.), Dengzi, Stn. 16, 25.vi.1961, (1 ex.), leg. K.C. Jayram.

**Distribution.** India: Arunachal Pradesh (West Kameng, Tawang), Assam, Himachal Pradesh, Jammu & Kashmir, Manipur, Punjab, Rajasthan, Sikkim, and West Bengal. Elsewhere: Bhutan, China, Japan, Nepal, and Pakistan (Poorani 2002b, 2004; Kovář 2007; Poorani & Sambath 2017).



**Figs 10–18.** Habitus of (10) *Coelophora bissellata* Mulsant, 1850; (11) *Halyzia dejavu* Poorani & Booth, 2006; (12) *Harmonia dimidiata* (Fabricius, 1781); (13) *Harmonia eucharis* (Mulsant, 1853); (14) *Harmonia sedecimnotata* (Fabricius, 1801); (15) *Hippodamia variegata* (Goeze, 1777); (16) *Illeis confusa* Timberlake, 1943; (17) *Illeis indica* Timberlake, 1943; (18) *Macroilleis hauseri* (Mader, 1930).

**16. *Harmonia eucharis* (Mulsant, 1853) (Fig. 13)***Ballia eucharis* Mulsant, 1853: 167.

**Material examined.** West Kameng: Rahung, Stn. 34, 7.iv.1961, (1 ex.), Rahung, Stn. 35, (1 ex.) 24.iv.1961, leg. K.C. Jayram. Tawang: Zemithang, 24.vi.2017, (1 ♀), leg. J. Saini. Dibang Valley, Dihang Dibang Biosphere Reserve, Mipi, 01.xi.2017 (1 ex), leg. D. Gupta.

**Distribution.** India: Arunachal Pradesh (Tawang, West Kameng, Dihang Dibang Biosphere Reserve), Jammu and Kashmir, Himachal Pradesh, Manipur, Sikkim, Uttarakhand, and Uttar Pradesh. Elsewhere: China, Myanmar, Nepal, and Pakistan (Poorani 2002b, 2004; Kovář 2007; Poorani & Sambath, 2017).

**Remarks.** See Kovář (2007) for synonyms.

**17. *Harmonia manillana* (Mulsant, 1866)***Caria manillana* Mulsant, 1866: 170.*Leis atrocincta* Mulsant, 1866: 175.*Neda paulinae* Mulsant, 1866: 203.*Leis dunlopi* Crotch, 1874: 121.*Leis cerasicolor* Crotch, 1874: 121.*Leis aterrima* Crotch, 1874: 121.*Leis papuensis* Crotch, 1874: 121.*Leis papuensis* var. *suffusa* Crotch, 1874: 121.

**Distribution.** India: Arunachal Pradesh (Pasighat). Elsewhere: Philippines, Malaysia, and Indonesia (Iablokoff-Khnzorian 1982; Poorani & Booth 2016).

**Remarks.** Poorani & Booth (2016) first time recorded this species from the Palearctic region of Arunachal Pradesh, and also mentioned to be very rare in mainland India. Earlier, the species was known from the oriental region of Indonesia, Malaysia, and the Philippines. We could not find specimen of this species from the study area in our collection.

**18. *Harmonia sedecimnotata* (Fabricius, 1801) (Fig. 14)\****Coccinella sedecimnotata* Fabricius, 1801: 370.*Daulis 16-notata*: Mulsant, 1850: 296.*Callineda sedecimnotata*: Crotch, 1874: 161.*Harmonia sedecimnotata*: Timberlake, 1943: 18.

**Material examined.** Changlang: Namdapha National Park, Deban, 24.vi.2017 (3 exs) (24918/H4A), leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Changlang), Sikkim, and West Bengal. Elsewhere: China, Nepal, and Southeast Asia (Poorani 2002b, 2004; Kovář 2007).

**Genus *Hippodamia* Chevrolat, 1836****19. *Hippodamia variegata* (Goeze, 1777) (Fig. 15)\****Coccinella variegata* Goeze, 1777: 246.*Adonia variegata*: Mulsant, 1846: 39.*Hippodamia variegata*: Belicek, 1976: 338.*Hippodamia* (*Adonia*) *variegata*: Iablokoff-Khnzorian, 1982: 326.

**Material examined.** West Kameng: Dengzi, Stn. 16, 25.v.1961 (2 exs); Salari, Stn. 60, 22.vi.1961 (2 exs), leg. K.C. Jayram.

**Distribution.** India: Arunachal Pradesh, Himachal Pradesh, Jammu & Kashmir, Maharashtra, and Uttar Pradesh. Elsewhere: widely distributed in Afrotropical, Nearctic, Oriental regions; Afghanistan, China, Nepal, and Pakistan (Poorani 2002b, 2004; Kovář 2007).

**Genus *Illeis* Mulsant, 1850****20. *Illeis confusa* Timberlake, 1943 (Fig. 16)\****Illeis confusa* Timberlake, 1943: 61.

**Material examined.** Tawang: Zemithang, 24.vi.2017 (1♂) (24909/H4A), leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Tawang), Assam, and West Bengal. Elsewhere: China, Nepal, and Thailand (Poorani 2002b, 2004; Poorani & Lalitha 2018; Kovář 2007).

**21. *Illeis indica* Timberlake, 1943 (Fig. 17)\****Illeis indica* Timberlake, 1943: 61.

**Material examined.** Tawang: Zemithang, 24.vi.2017 (1 ex.), leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Tawang), Jammu & Kashmir, Himachal Pradesh, and Uttar Pradesh. Elsewhere: Pakistan, and Thailand (Poorani 2002b, 2004; Kovář 2007).

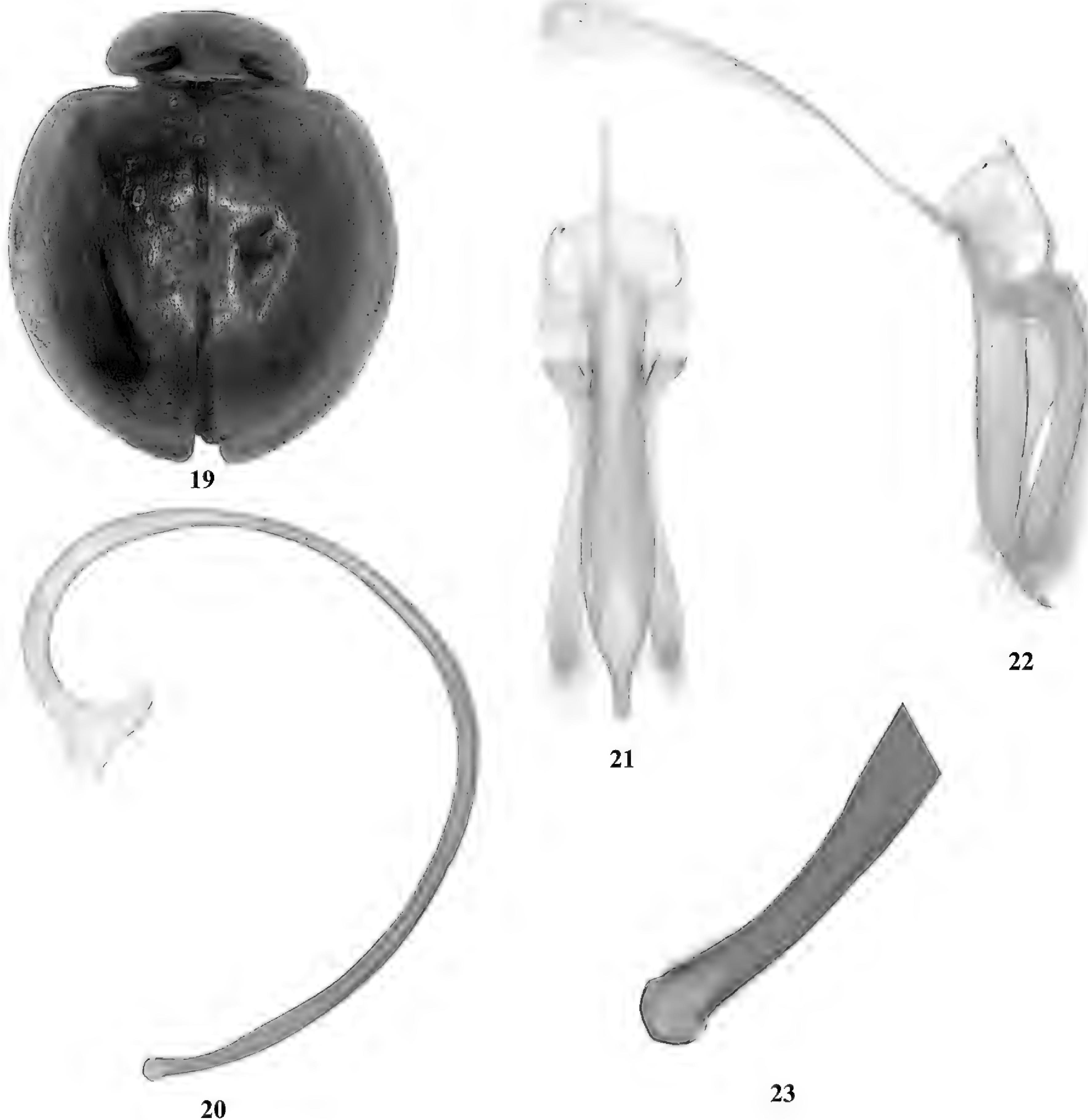
**22. *Illeis shensiensis* Timberlake, 1943 (Figs 19–23)\*\***

*Illeis shensiensis* Timberlake, 1943: 61; Bielawski, 1961: 358, figs 5–6, 14; Ren et al., 2009: 243.

**Material examined.** Changlang: Deban, 355 m, 24.xii.2017 (2 ♂♂) (25409/H4A), leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Changlang). Elsewhere: China (Shensi), and Pakistan (Kovář 2007; Hayat et al. 2017).

**Remarks:** *I. shensiensis* can be distinguished from closely related species by the following characters: pro-



**Figs 19–23.** *Illeis shensiensis* Timberlake, 1943. (19) Habitus; (20) Siphon; (21) Phallobase in dorsal view; (22) Phallobase in lateral; (23) Apical portion of Siphon.

notum with two small black marks (Fig 19), siphon not bifid at apex (Fig 23), median lobe of tegmen having apex strongly curved upward and more or less depressed (Figs 21, 22), parameres shorter and stouter and somewhat strongly curved at base, otherwise straight (Fig 21). The male genitalia of our specimen is identical with that of *I. shensiensis* as illustrated in Bielawski (1961: 358, Figs 5–6, 14) and Ren et al. (2009: 243, Fig 325).

#### Genus *Macroilleis* Miyatake, 1965

**23. *Macroilleis hauseri* (Mader, 1930) (Fig. 18)**  
*Halyzia hauseri* Mader, 1930: 162.  
*Macroilleis hauseri*: Miyatake 1965: 71–73.

**Material examined.** Tawang: Zemithang, 24.vi.2017 (1 ♂) (25399/H4A), leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Tawang), and West Bengal. Elsewhere: Bhutan, China, Vietnam, and

Pakistan (Poorani 2002b; 2004; Kovář 2007; Poorani & Sambath 2017).

**Genus *Menocheilus* Timberlake, 1943**

**24. *Menocheilus sexmaculata* (Fabricius, 1781)**

(Fig. 24)

*Coccinella sexmaculata* Fabricius, 1781: 96.

**Material examined.** Papum Pare: Bandardewa, 11.iv.2001 (2exs) (24307/H4A), leg. Sheela. Dihang Dibang Biosphere Reserve, Maliny, 25.x.2017 (2 exs) (24438/H4A), leg. D. Gupta.

**Distribution.** Widely distributed in India including Arunachal Pradesh (Papum Pare; Dihang Dibang Biosphere Reserve). Elsewhere: Australian region, Arab Emirates, Afghanistan, Bangladesh, Bhutan, China, Indonesia, Iran, Japan, Malaysia, Myanmar, Nepal, Oman, Pakistan, Sri Lanka, Philippines, and Vietnam (Poorani 2002b, 2004; Kovář 2007).

**Remarks.** See Kovář (2007: 619) for synonymy.

**Genus *Micraspis* Chevrolat, in Dejean, 1836**

**25. *Micraspis univittata* (Hope, 1831) (Fig. 25)\***

*Coccinella univittata* Hope, 1831: 31.

*Alesia univittata*: Mulsant, 1850: 357.

*Tytthaspis univittata*: Korschefsky, 1932: 384.

*Micraspis univittata*: Iablokoff-Khnzorian, 1982: 511.

**Material examined.** Along: Hissan Colony, 11.vi.2000 (1 ex.) (25408/H4A), leg. R.S. Mridha.

**Distribution.** India: Arunachal Pradesh (Along), Andhra Pradesh, Bihar, Karnataka, Odisha, Tamil Nadu, Tripura, and Uttarakhand. Elsewhere: China, and Nepal (Poorani 2002b; 2004; Kovář 2007).

**26. *Micraspis unicus* Poorani, 2019**

*Micraspis unicus* Poorani, 2019: 190, fig. 1.

**Distribution.** India: Arunachal Pradesh (Mayodia).

**Remarks.** This species was described recently by Poorani (2019) from Arunachal Pradesh, based on male, holotype and female paratype, deposited at NBAIR. We could not find any specimen of this species in our collection.

**Genus *Oenopia* Mulsant, 1850**

**27. *Oenopia chinensis* (Weise, 1912)**

*Coelophora chinensis* Weise, 1912: 113.

*Gyrocaria chinensis*: Miyatake, 1965: 65.

*Oenopia chinensis*: Hoàng, 1983: 91.

**Distribution.** India: Arunachal Pradesh (Pasighat), and Meghalaya. Elsewhere: China (Poorani 2002a, 2002b; 2004; Kovář 2007; Poorani & Thangjam 2019).

**Remarks.** Earlier known from China, this species was reported from India by Poorani & Thangjam (2019), based on a female specimen from Meghalaya and larvae from Arunachal Pradesh, materials of which are deposited at NBAIR. We could not find any adult specimen of this species from the study area in our collection.

**28. *Oenopia kirbyi* Mulsant, 1850 (Fig. 26)\***

*Oenopia kirbyi* Mulsant, 1850: 425; Poorani, 2002a: 102, figs 4, 16, 23, 31.

*Gyrocaria kirbyi*: Miyatake, 1965: 66, fig. 34.

**Material examined.** Dihang Dibang Biosphere Reserve: Maliney, 27.x.2017 (1ex.) (24432/H4A); Anini, 27.x.2017 (1ex.) (24433/H4A), leg. D. Gupta. Siang: NEFA, 10.xi.1971 (1 ex. on leaves) (25404/H4A), leg. S. Ghose. Roing: Dibang Valley, 250 m, 25.i.2000 (2 exs) (25405/H4A), 27.i.2000 (1 ex.) (25406/H4A), 29.i.2000 (5 exs) (25407/H4A), leg. S.K. Mondal.

**Distribution.** India: Arunachal Pradesh (Dihang-Dibang Biosphere Reserve, Siang, Roing), Meghalaya, Mizoram, Sikkim, and West Bengal; Bhutan. Elsewhere: China, Myanmar, Nepal, and Thailand (Poorani 2002a, 2002b, 2004; Kovář 2007).

**29. *Oenopia mimica* Weise, 1902 (Fig. 27)\***

*Oenopia mimica* Weise, 1902: 505; Poorani, 2002a: 104, figs 5, 17, 24, 32.

*Gyrocaria mimica*: Miyatake, 1985: 16.

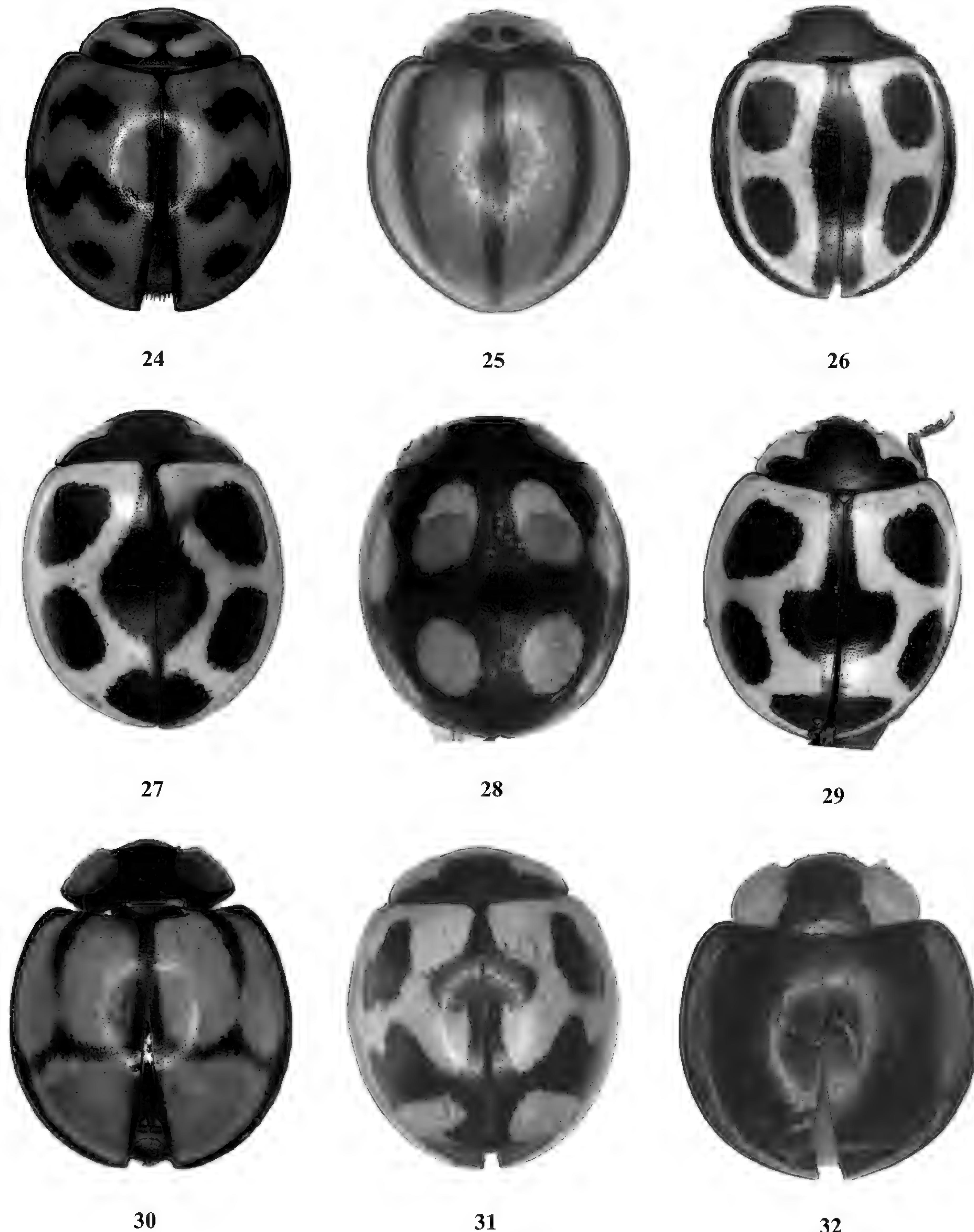
**Material examined.** Siang: NEFA, 10.xi.1971 (1 ex. on leaves) (25324/H4A), leg. S. Ghose. Lower Subansiri: Bandardewa, 19.x.1996 (1 ex.) (25325/H4A), leg. S.K. Mondal. Tawang: Jung, 23.ix.2013 (2 exs) (25323/H4A), leg. J. Majumder. Dihang Dibang Biosphere Reserve, Anini, 25.x.2019 (1 ex.), leg. D. Gupta.

**Distribution.** India: Arunachal Pradesh (Tawang, Siang, Lower Subansiri), Himachal Pradesh, Uttar Pradesh, and Sikkim. Elsewhere: Laos, Myanmar, and Nepal (Poorani 2002a, 2002b, 2004; Kovář 2007).

**30. *Oenopia quadripunctata* Kapur, 1963 (Fig. 28)\***

*Oenopia quadripunctata* Kapur, 1963: 27; Poorani, 2002a: 102, fig. 3.

**Material examined.** Debang Valley: Old Aloppa, 16.ix.2000 (1 ex.), leg. A.R. Lahiri.



**Figs 24–32.** Habitus of (24) *Menocheilus sexmaculata* (Fabricius, 1781); (25) *Micraspis univittata* (Hope, 1831); (26) *Oenopia kirbyi* Mulsant, 1850; (27) *Oenopia mimica* Weise, 1902; (28) *Oenopia quadripunctata* Kapur, 1963; (29) *Oenopia sauzeti* Mulsant, 1866; (30) *Oenopia sexareata* (Mulsant, 1853); (31) *Propylea luteopustulata* (Mulsant, 1850); (32) *Synona melanopepla* (Mulsant, 1850).

**Distribution.** India: Arunachal Pradesh (Debang Valley), Meghalaya, Mizoram, Nagaland, Sikkim, and West Bengal. Elsewhere: Bhutan, China, Myanmar, and Nepal (Poorani 2002a, 2002b, 2004; Kovář 2007).

### 31. *Oenopia sauzeti* Mulsant, 1866 (Fig. 29)

*Oenopia sauzeti* Mulsant, 1866: 281; Poorani, 2002a: 103, figs 6, 18, 25, 33.

*Gyrocaria sauzeti*: Miyatake, 1967: 76; 1985: 15, figs 30–33.

**Material examined.** Lower Subansiri: Bandardewa, 25.i.2000 (1 ex.) (24295/H4A), leg. S.K. Mondal. West Kameng, Kalaktang, Stn.17, 17.iii.1961 (1 ex.), Dukongko River, 02.v.1961 (1 ex.); Rupa, Stn. 29, 03.v.1961 (1 ex.); Shergaon, Stn. 51, 5.v.1961 (1 ex.)/Stn. 25, 8.v.1961 (2 exs); Domko, Stn. 52, 10.v.1961 (1 ex.); Moshing, Stn. 22, 11.v.1961 (1 ex.); Dengzi, Stn. 16, 25.v.1961 (3 exs); Ankaling, Stn. 12, 25.v.1961 (1 ex.), leg. K.C. Jayram. Changlang: Namdapha National Park, 361m, 24.vi.2017 (1 ex.) (24855/H4A), leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Changlang; Namdapha National Park, West Kameng, Lower Subansiri), Assam, Himachal Pradesh, Jammu & Kashmir, Meghalaya, Sikkim, Uttar Pradesh, and West Bengal. Elsewhere: Bhutan, China, Pakistan, Laos, Myanmar, Nepal, Thailand, and Vietnam (Poorani 2002a, 2002b, 2004; Kovář 2007; Poorani & Sambath 2017).

### 32. *Oenopia sexareata* (Mulsant, 1853) (Fig. 30)

*Coelophora sexareata* Mulsant, 1853: 181; Poorani, 2002a: 101, figs 2, 15, 22, 30.

*Coelophora sexareata* var. *lacerata* Sicard, 1913: 500.

*Gyrocaria sexareata*: Miyatake, 1967: 76.

*Oenopia sexareata*: Hoàng, 1983: 62, 92.

**Material examined.** West Kameng: Rahung, Stn.35, 24.iv.1961, (1 ex.), Siggun, Stn.50, 4.v.1961, (3 exs), Denzi, Stn.16, 25.v.1961, (1 ex.) leg. K.C. Jayram, Lower Subansiri: Banderdewa, 19.x.1996 (1 ex.) (25327/H4A), leg. A.M. Biswas. Roing: Dibang Valley, 250m, 25.i.2000 (1 ex.) (25328/H4A), 29.i.2000 (3 exs) leg. S.K. Mondal. Papum Pare: Bandardewa, 11.iv.2001 (2 exs) (25329/H4A) leg. Sheela. Dihang Dibang Biosphere Reserve: Maliney, 27.x.2017 (1ex.) (24434/H4A) leg. D. Gupta.

**Distribution.** India: Arunachal Pradesh (Lower Subansiri, Roing, Papum Pare, West Kameng, Dihang Dibang Biosphere Reserve), Assam, Bihar, Himachal Pradesh, Meghalaya, Sikkim, Uttar Pradesh, and West Bengal. Elsewhere: Bhutan, China, Indonesia, Japan, Myanmar,

Nepal, and Vietnam (Canepari 1997; Poorani 2002a, 2002b, 2004; Kovář 2007).

### Genus *Propylea* Mulsant, 1846

#### 33. *Propylea luteopustulata* (Mulsant, 1850) (Fig. 31)

*Oenopia (Pania) luteopustulata* Mulsant, 1850: 421.

*Propylea luteopustulata*: Vandenberg & Gordon, 1991: 30.

**Material examined.** West Kameng: Kalaktang, Stn.17, 17.iii.1961, (1 ex.); Rahung, Stn. 35, 24.iv.1961, (1 ex.); Rahung, Stn. 34, 25.iv.1961, (1 ex.); Siggun, Stn. 50, 4.v.1961, (1 ex.); Shergaon, Stn. 25, 08.v.1961 (1 ex.); Domko, Stn. 52, 10.v.1961, (1 ex.); Moshing, Stn. 22, 11.v.1961 (1 ex.); Dengzi, Stn.16, 15.v.1961, (1 ex.); Ankaling, Stn. 12, 15.v.1961 (2 exs), leg. K.C.Jayram; Zamiri, 14.x.1997 (1 ex.), leg. S.K. Mondal. Siang: NEFA, 10.xi.1971 (2 exs on leaves), leg. S. Ghose. Lower Subansiri: Bandardewa, 19.x.1996 (2 exs), leg. A.M. Biswas. Tawang: Center Dirang, 15.xi.1997 (2 exs on foliage), leg. A.K. Sanyal. Roing: Debang Valley, Rukmo, 27.i.2000 (2 exs), leg. S.K. Mondal. Changlang: Namdapha National Park, 361m, 24.vi.2017 (1 ex.), leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Siang, Lower Subansiri, Tawang, West Kameng, Roing, Changlang), Assam, Andaman & Nicobar Islands, Himachal Pradesh, Meghalaya, Uttar Pradesh, Sikkim, and West Bengal. Elsewhere: Bhutan, China, Myanmar, Nepal, Sri Lanka, Thailand, and Vietnam (Poorani 2002b, 2004; Kovář 2007; Poorani & Sambath 2017).

### Genus *Synona* Pope, 1989

#### 34. *Synona melanopepla* (Mulsant, 1850) (Fig. 32)\*

*Synia melanopepla* Mulsant, 1850: 376.

*Synia melanaria* ab. *melanopepla*: Korschefsky, 1932: 276.

*Leis rougeti* Mulsant, 1866: 175.

*Synia melanaria* ab. *rougeti*: Korschefsky, 1932: 276.

*Synona melanopepla*: Poorani et al., 2008: 583, figs 1, 2, 17–22, 52, 53.

**Material examined.** Roing: Dibang Valley, 19.ix.2000 (1 ♂) (25326/H4A), leg. R.S. Mridha.

**Distribution.** India: Arunachal Pradesh (Roing), Assam, Bihar, Karnataka, Meghalaya, Odisha, Tamil Nadu, and Uttar Pradesh. Elsewhere: Vietnam (Kovář 2007; Poorani et al. 2008).

**Tribe Epilachnini Mulsant, 1846****Genus *Afissa* Dieke, 1947**

Tomaszewska & Szawaryn (2016) in their revision of world genera of Epilachnini, proposed *Epilachna* to be a new world genus and established *Afissa* as a valid genus. Here, three species *Epilachna gibbera* Crotch, 1874, *Epilachna mystica* Mulsant, 1850, and *Epilachna undecimspilota* Hope, 1831 are combined with *Afissa* based on the shared morphological characters, proposed by Tomaszewska & Szawaryn (2016) for the genus: Lateral margins of elytra not or hardly visible dorsally, sometimes narrowly explanate, and meta-ventral and abdominal post-coxal lines present.

**35. *Afissa gibbera* (Crotch, 1874) comb. nov. (Fig. 33)\***

*Epilachna gibbera* Crotch, 1874: 80.

*Afissa gibbera*: Kapur, 1963: 10.

*Epilachna gibbera*: Jadwiszczak & Wegrzynowicz, 2003: 69.

**Material examined.** Tawang: Camp, Stn. No., 14.xii.1985, (1 ♂), leg. S.K.B. & ANTJ.

**Distribution.** India: Arunachal Pradesh (Tawang), and Sikkim. Elsewhere: Nepal (Poorani 2004).

**36. *Afissa mystica* (Mulsant, 1850) comb. nov. (Figs 41–50)**

*Epilachna mystica* Mulsant, 1850: 841.

*Afissa mystica*: Dieke, 1947: 146, figs 100, 169.

*Epilachna mystica*: Li & Cook, 1961: 51.

**Material examined.** West Kameng: Munna, 8500 m, 10.x.1996, (4 exs), leg. S.K. Mondal; Bomdila: Sherra Bash, 30.viii.1998, (3 exs), leg. A.R. Lahiri. East Kameng: Seppo, 3500m, 11.x.1996, (2 exs), leg. S.K. Mondal.

**Distribution.** India: Arunachal Pradesh (Tawang, West Kameng, East Kameng), Karnataka, Sikkim, Uttarakhand, and West Bengal (North). Elsewhere: Bhutan, China, Myanmar, and Nepal (Poorani 2004; Kovář 2007; Poorani & Sambath 2017).

**37. *Afissa nielamuensis* (Pang & Mao, 1977) (Fig. 34)**

*Epilachna nielamuensis* Pang & Mao, 1977: 323, 327; Miyatake, 1985: 30; Jadwiszczak & Wegrzynowicz, 2003: 94; Ren et al., 2009: 291.

*Afissa nielamuensis*: Poorani & Thangjam, 2019: 7, figs 6A–B.

**Material examined.** Tawang: Jang, Jangda, 2,572 m, 24.ix.2018 (1 ♂), leg. J. Saini.

**Distribution.** India: Arunachal Pradesh (Tawang). Elsewhere: China and Nepal (Poorani 2004; Poorani & Thangjam 2019).

**38. *Afissa rana* (Kapur, 1958) (Figs 51–53)\*\***

*Afissula rana* Kapur, 1958: 320.

*Afissa rana*: Tomaszewska & Szawaryn, 2016: 53.

**Material examined.** West Kameng: Bomdila, Stn. 32, 25.vi.1961 (6 exs), 29.vi.1961 (1 ex.), Dukongko River, Stn. 49, 02.v.1961 (2 exs), Moshing, Stn. 22, 03.vi.1961 (3 exs), leg. K.C. Jayram; Bomdila, Tinga, 29.vi.1995 (7 exs) (25652/H4A), Sherra Bash, 30.viii.1998 (2 exs) (25653/H4A).

**Type material.** Paratype: B.M. Nepal Expedition, 1949 (B.M. 1949–637), *Afissula rana* gen.n., sp. nov., A.P. Kapur Det., 1954 [ZSI Registration Number: 9983/H4].

**Distribution.** India: Arunachal Pradesh (West Kameng). Elsewhere: China, and Nepal (Poorani 2004).

**Remarks.** *A. rana* can be distinguished from closely related species by the following characters: lateral margins of elytra invisible in dorsal view, pronotum reddish-testaceous except for yellowish margins (Fig 51), siphon narrow and lancet-shaped at apex (Fig 53), and parameres shorter than median lobe (Fig 52). The male genitalia of our specimen is identical with that of *A. rana* as illustrated in the original description by Kapur (1958: 320, figs 5a, c–f). The paratype of the species NZSI was also examined.

**39. *Afissa undecimspilota* (Hope, 1831) comb. nov. (Fig. 35)\***

*Coccinella 11-spilota* Hope, 1831: 31.

*Epilachna undecimspilota*: Jadwiszczak & Wegrzynowicz, 2003: 125.

**Material examined.** West Kameng: Dirang, Rahung, 1830m, 16.viii.1961, (2 exs), leg. S. Biswas.

**Distribution.** India: Arunachal Pradesh (West Kameng), and Northern India. Elsewhere: China, Bhutan, and Nepal (Poorani 2004; Kovář 2007).

**Genus *Diekeana* Tomaszewska & Szawaryn 2015****40. *Diekeana macularis* (Mulsant, 1850) (Fig. 36)\***

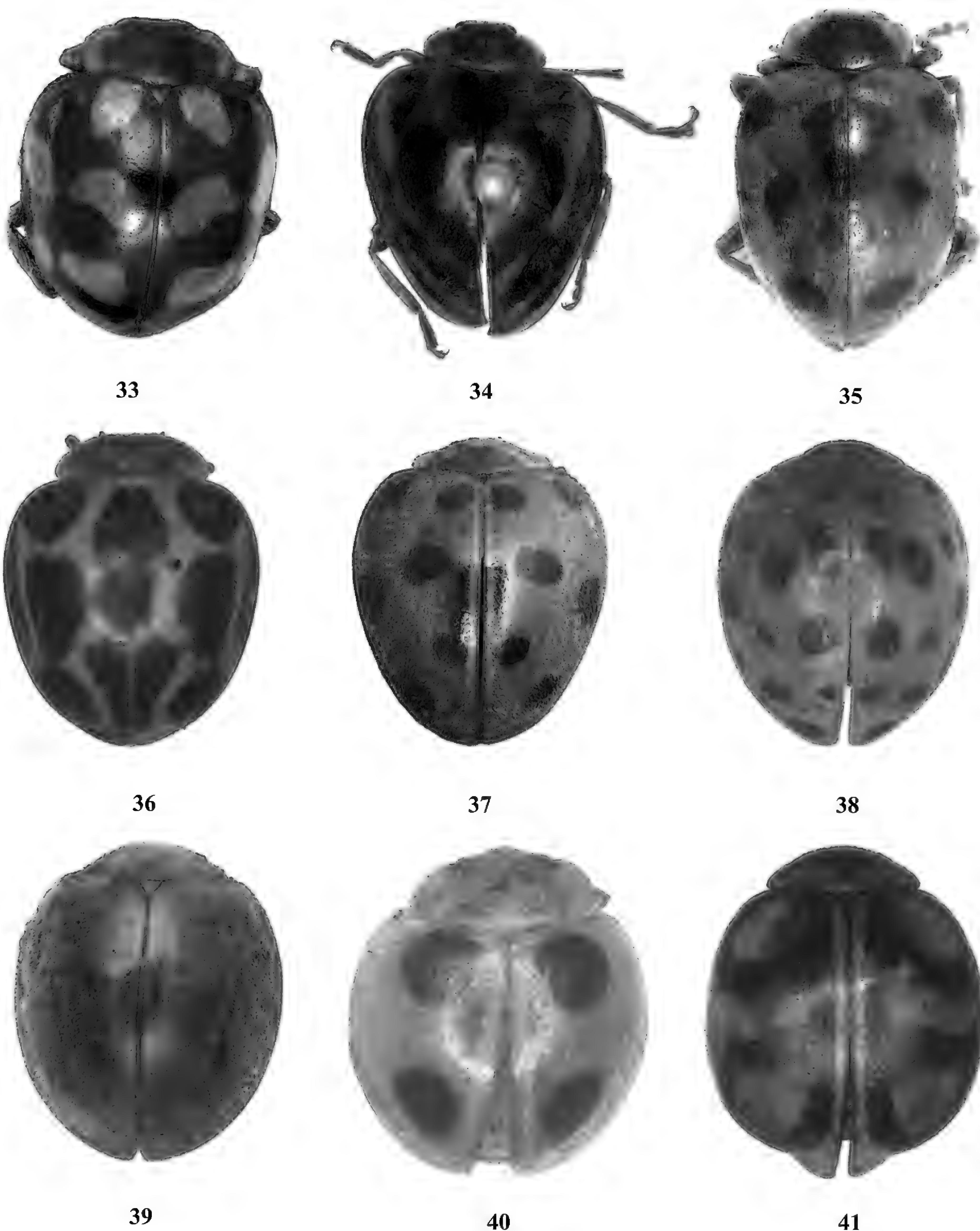
*Epilachna macularis* Mulsant, 1850: 797.

*Solanophila macularis* ab. *donckieri* Weise, 1912: 112.

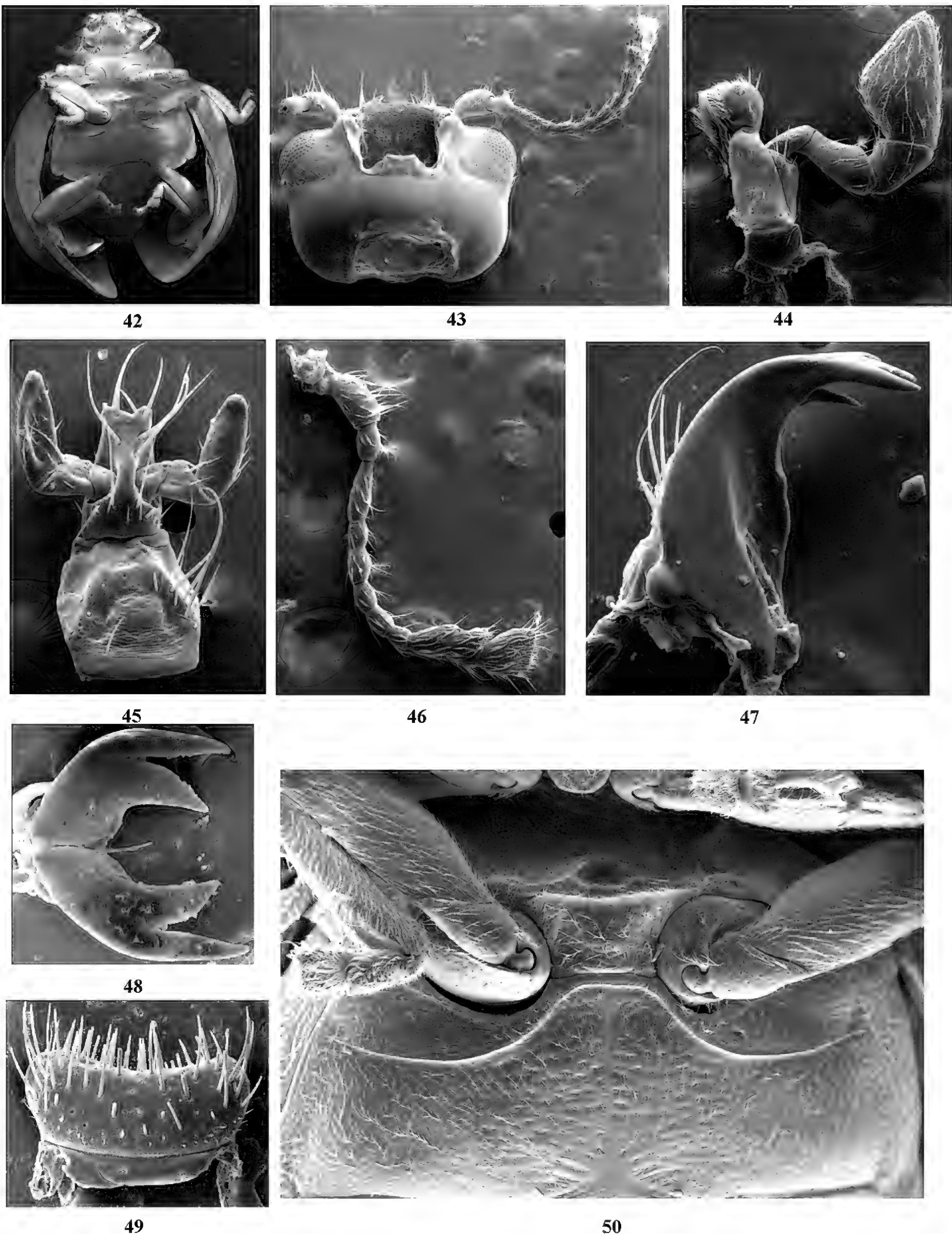
*Afissa macularis*: Dieke, 1947: 120, figs 78, 153.

*Epilachna macularis*: Jadwiszczak & Wegrzynowicz, 2003: 86.

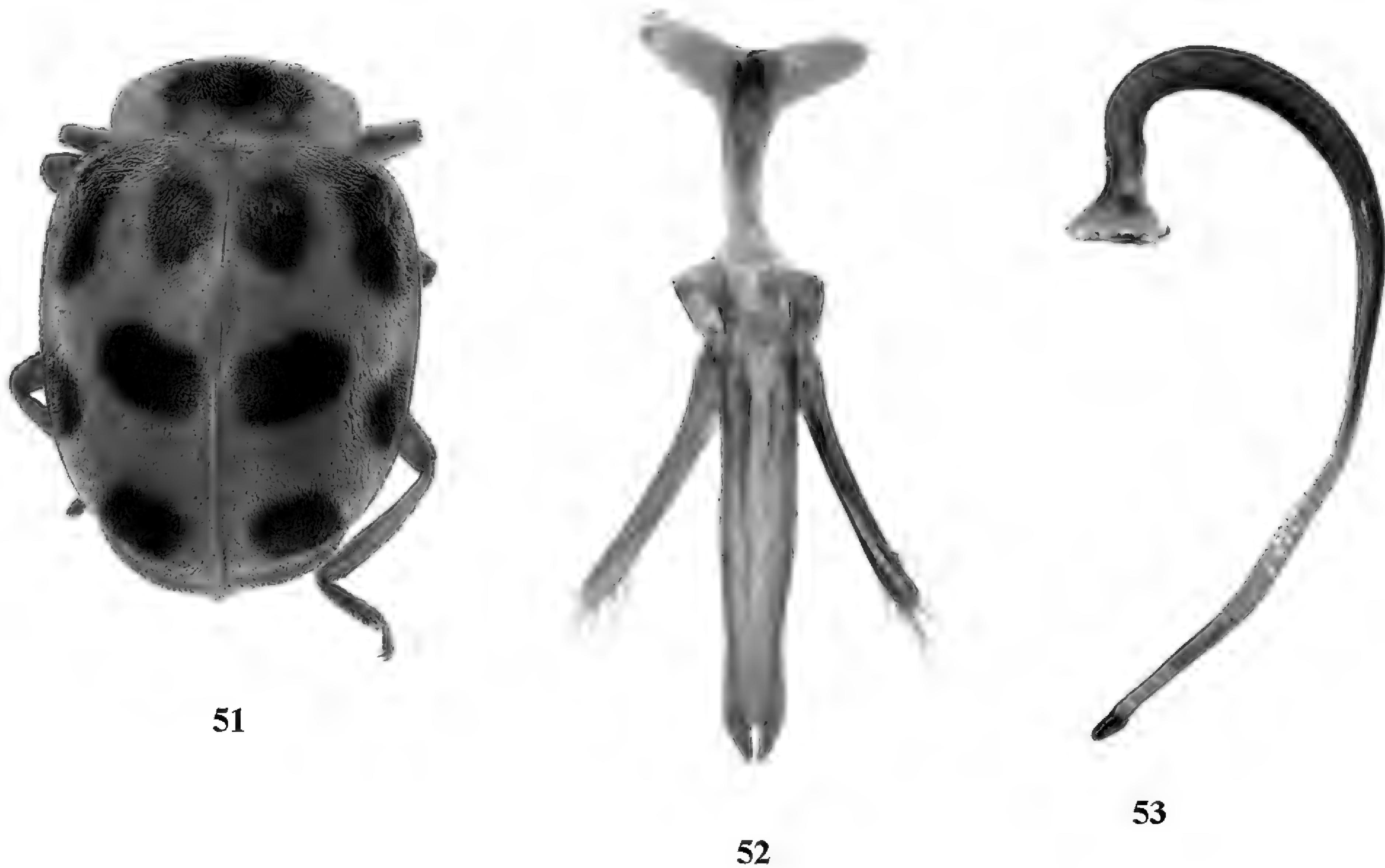
*Diekeana macularis*: Tomaszewska & Szawaryn, 2016: 74.



**Figs 33–41.** Habitus of (33) *Afissa gibbera* (Crotch, 1874) comb. nov.; (34) *Afissa nielamuensis* (Pang & Mao, 1977); (35) *Afissa undecimspilota* (Hope, 1831) comb. nov.; (36) *Diekeana macularis* (Mulsant, 1850); (37) *Henosepilachna indica* (Mulsant, 1850); (38) *Henosepilachna vigintioctomaculata* (Motschulsky, 1857); (39) *Rodolia fumida* Mulsant, 1850; (40) *Jauravia quadrinotata* Kapur, 1946; (41) *Afissa mystica* (Mulsant, 1850) comb. nov.



**Figs 42–50.** Scanning electron microscope images of *Afissa mystica* (Mulsant, 1850) comb. nov. (42) Habitus in ventral view; (43) Head in ventral view; (44) Maxilla; (45) Labium; (46) Antennae; (47) Mandible; (48) Tarsal claws; (49) Labrum; (50) Meso-thorax.



**Figs 51–53.** *Afissa rana* (Kapur, 1958). (51) Habitus; (52) Phallobase in dorsal view; (53) Siphon.

**Material examined.** Lohit: Dapha Bum, Kamlang River, 3020 ft, Stn. No. 23, 22.xii.1969, (4 exs), leg. J.M. Julka. Tawang: Bomdila, 29.ix.2013 (1 ex. from *Astimicia* sp.) (25410/H4A), leg. J. Majumder.

**Distribution.** India: Arunachal Pradesh (Lohit, Tawang), and Meghalaya. Elsewhere: China and Nepal (Dieke 1947; Jadwiszczak & Wegrzynowicz 2003; Poorani 2004; Kovář 2007).

#### Genus *Henosepilachna* Li, 1961

##### 41. *Henosepilachna indica* (Mulsant, 1850) (Fig. 37)\*

*Epilachna indica* Mulsant, 1850: 776.

*Epilachna ceylonica* Weise, 1901: 418.

*Epilachna indica*: Kapur, 1961: 133–140.

*Epilachna tertia* Dieke, 1947: 66.

*Henosepilachna indica*: Jadwiszczak & Wegrzynowicz, 2003: 154.

**Material examined.** Lower Subansiri: Tamen, 457m, Stn. No. 17, 18.v.1966 (1 ex.), leg. A.N.T. Joseph. Lohit: Deopani, 350m, Stn. No. 8, 6.iii.1969, (3 exs), leg. S.K. Tandon; Kandu, 300 m, Stn. No. 9, 7.iii.1969, (5 exs); Digaru Road, 150m, Stn. No. 12, 11.iii.1969 (2 exs); Hayaliaung Road, 700 m, Stn. No. 13, 12.iii.1969,

(4 exs); Lohitpur Road, 150m, Stn. No. 14, 13.iii.1969 (1 ex.); Namsai, 100 m, Stn. No. 15, 15.iii.1969, (1 ex.), leg. S.K. Tandon.

**Distribution.** India: Arunachal Pradesh (Lower Subansiri, Lohit), Assam, and West Bengal. Elsewhere: Bhutan, China, Laos, Myanmar, Nepal, and Vietnam (Dieke 1947; Poorani 2004; Kovář 2007).

##### 42. *Henosepilachna vigintioctomaculata* (Motschulsky, 1857) (Fig. 38)\*\*

*Epilachna vigintioctomaculata* Motschulsky, 1857: 40.

*Epilachna 28-maculata* a. *incompleta* Mader, 1930: 184.

*Epilachna 28-maculata* a. *coalescens* Mader, 1930: 184.

*Henosepilachna vigintioctomaculata*: Jadwiszczak & Wegrzynowicz, 2003: 178.

**Material examined.** NEFA, Abor, 29.xii.1911, (1 ex.) leg. S.W. Kemp, New Aloppa: Rang, 17.ix.2000 (4 exs) (25332/H4A), leg. A.R. Lahiri.

**Distribution.** India: Arunachal Pradesh. Elsewhere: China, Japan, North Korea, Nepal, Russia, and Vietnam (Katakura 1981; Poorani 2004).

**Remarks.** This is the first verified record of the species from India, though it has been included from India by Poorani (2004).

**Tribe Noviini Mulsant, 1846**

**Genus *Rodolia* Mulsant, 1850**

**43. *Rodolia fumida* Mulsant, 1850 (Fig. 39)\***

*Rodolia fumida* Mulsant, 1850: 904.

*Rodolia roseipennis* Mulsant, 1850: 904.

*Rodolia chermesina* Mulsant, 1850: 905.

*Epilachna arethusa* Mulsant, 1853: 254.

*Epilachna testicolor* Mulsant, 1853: 255.

**Material examined.** West Kameng: Ankaling, Stn.11, 17.v.1961 (1 ex.), leg. K.C. Jayram.

**Distribution.** India: Arunachal Pradesh (West Kameng), Assam, Bihar, Delhi, Gujarat, Himachal Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Tamil Nadu, Uttarakhand, Uttar Pradesh, and West Bengal. Elsewhere: China, Myanmar, Pakistan, and Sri Lanka (Kapur 1949; Poorani 2004; Kovář 2007).

**Tribe Sticholotidini Weise, 1901**

**Genus *Jauravia* Motschulsky, 1858**

**44. *Jauravia quadrinotata* Kapur, 1946 (Fig. 40)\***

*Jauravia quadrinotata* Kapur, 1946: 85; Miyatake, 1985: 3, figs 1–3.

**Material examined.** West Kameng: 15.iii.1961 (1 ex.), leg. K.C. Jayram.

**Distribution.** India: Arunachal Pradesh (West Kameng), Assam, Meghalaya, Sikkim, and West Bengal. Elsewhere: Bhutan, China, and Nepal (Bielawski 1972; Canepari 1997; Poorani 2002b, 2004; Kovář 2007).

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## Research article

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# Diversity of bats (Mammalia: Chiroptera) along an altitudinal gradient in the western region of Cameroon

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**Abstract.** We investigated the patterns of bat species richness, abundance and distribution along an altitudinal gradient in the West region of Cameroon from December 2016 to November 2018 with the use of mist nets. Captures were conducted at 32 sites distributed over six distinct elevational ranges, comprising five sites in elevation range I (< 750 m a.s.l.), four sites in elevation range II (750–1,000 m a.s.l.), eight sites in elevation range III (1,000–1,250 m a.s.l.), six sites in elevation range IV (1,250–1,500 m a.s.l.), six sites in elevation range V (1,500–1,750 m a.s.l.) and two sites in elevation range VI (> 1,750 m a.s.l.). A total of 442 bats were captured during 95 sampling nights, comprising 25 species, 16 genera and six families. Out of the 25 species, *Myonycteris angolensis* was the most abundant species captured with 80 individuals, followed by *Micropteropus pusillus* (61 individuals) and *Eidolon helvum* (60 individuals). Moreover, species richness peaked at the mid-elevation range III (1,000–1,250 m a.s.l.), with 13 species, with richness decreasing both at higher and lower elevations. Elevation range I had the second highest species richness with 12 species, while elevational range VI had the least species richness with three species. Species abundance peaked at elevation range IV (750–1,250 m a.s.l.) and decreases at higher elevations. The sample efficiency was estimated as 72.8% and a species accumulation curve of bats did not reach an asymptote, indicating that our sampling was incomplete. Our data showed that species richness and abundance is affected by elevation, with species richness probably depending on habitat types and availability of resources such as food and suitable roost sites. Our data also contributes to a better description of the local fauna and fills gaps on the species distribution for high altitude sites.

**Key words.** Bat, elevation range, diversity, West region, Cameroon, altitudinal gradient.

## INTRODUCTION

The western region of Cameroon is characterized by a heterogeneous landscape, of series of high plateaus formed by volcanic massifs, the most important of which are the Bamboutos Mountains (2,740 m a.s.l.), interspersed among lowland areas such as the Nkam and Noun valleys, and the Tikar Plain (Temgoua 2011). This heterogeneous landscape composed of montane forest remnants, grassland savannah and gallery forest in valleys is part of the Mount Cameroon-Bioko ecoregion. This region harbors some of the most threatened ecosystems in the country (Temgoua 2011). Indeed, very little of the region's native biota remained, a consequence of decades of deforestation to make way for agriculture and urbanization. Moreover, the region harbors the highest population density of the country with 13% of the national population, concentrated in only 3% of the territory (MINEPAT 2010). The consequences of these strong anthropic pressures are

particularly evident on the Western High Plateau, where poor soil and low rainfall have aggravated the effects of deforestation, converting the area to grassland (Temgoua 2011). As pointed out by Estrada & Coates-Estrada (2002), human-induced land use changes, due to agriculture and urbanization are known to alter bat assemblages, depending on the functional identity of bat species.

Bats are a species-rich group of mammals with about 357 species recorded in Africa (ACR 2018). They are found in every biome throughout the African continent with the exception of some extremely hot regions, and the tops of high mountains (Happold & Happold 2013). Several authors suggest that species richness and distribution of bats is influenced by the availability of resources such as drinking sites, food and suitable roost (Curran et al. 2012; Happold & Happold 2013). The potential distribution of each bat species can be affected by these ecological variables. In the tropics, vegetation types correlated with altitude are the principal factors that deter-

mine bat species distribution (Kaňuch & Krištín 2006; Weier et al. 2016). Mammalian species show varied responses to altitudinal gradient, among which two patterns standout for bats: a clinal pattern in which species richness is higher in lower elevations and decreases with altitude (Graham 1983, 1990; Patterson et al. 1996; Pin-ares 2006; Flores-Saldana 2008; Peters et al. 2016; Peters et al. 2019), or modal pattern with a peak in richness at mid-altitudes (Sanchez-Cordero 2001; McCain 2005). In addition, Curran et al. (2012), recorded some bat species mainly at higher altitudes, mid-altitudes and others preferred both low and high altitudes. They further pointed out that bat activity and capture rates in the tropics are greater at lower altitudes because water is not a limiting factor.

Although many surveys have focused on the mammalian fauna of Cameroon, few of these studies concerned bats until recently. To the best of our knowledge none of these studies assessed how bat species richness varies with altitude in heterogeneous landscapes such as the West region of Cameroon. Moreover, bats are not included in conservation and wildlife management programs in tropical ecosystems despite their ecological and economic importance (Bakwo Fils 2009, 2010). This lack of ecological information about the bats fauna of Cameroon hinders any development and implementation of conservation strategies (Bakwo Fils 2010).

In the western region of Cameroon elevations reach as high as 2,000 m a.s.l. in some areas such as the Bamboutos and dip as low as 500 m a.s.l. in others such as the Noun and Nkam valleys. To our knowledge, bat diversity along an altitudinal gradient in the region has never been studied. This study provides novel data for the West region of Cameroon, a region poorly surveyed for bats. Furthermore, knowledge of species-landscape relationship and species distribution is essential for proper planning and efficient management of biodiversity (Jaberg & Guisan 2001).

The present study aims to investigate patterns of distribution of bat species, species richness and abundance along an elevational gradient in the western region of Cameroon. We hypothesize that altitudinal pattern of bat assemblages in the region would vary with altitude and habitat heterogeneity, and that abundance and species richness would decrease with increasing elevation.

## MATERIALS AND METHODS

### Study site

This study was conducted in the western region of Cameroon. The region is situated between 5° and 6° N and 10° and 11°30' E. The region covers a total surface area of 13.892 km<sup>2</sup> (Olivry 1975; Brenac 1988). The vegetation is principally woodland savannah of the Sahel type,

interspersed among open dry forest. Very little of the natural vegetation still exists because deforestation has turned most of the area into grassland (Temgoua 2011). The climate of the western region of Cameroon is of the Equatorial, Guinean type characterized by two major seasons: a rainy season from mid-March to November with peak precipitation in August and a dry season from December to April. The annual rainfall varies between 1,000 mm and 2,000 mm depending on the year (Rivière 2000). The average annual temperature varies between 21.3°C and 29°C (Brenac 1988).

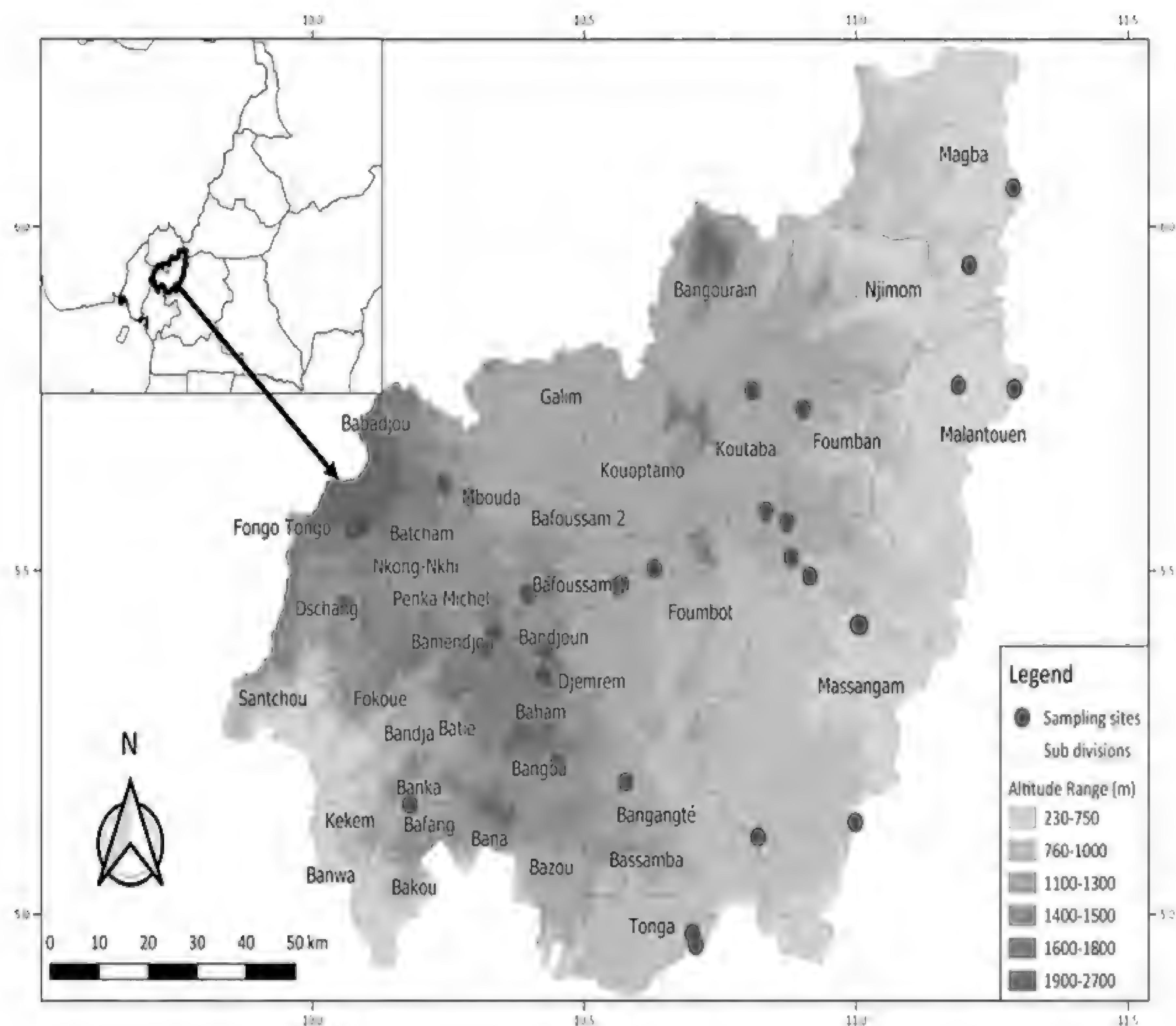
### Bats capture and identification

The survey was conducted from December 2016 to November 2018 at different altitudinal ranges (Fig. 1). Sampling was conducted over 95 nights across 32 sites. Bat activity was investigated in six different elevational ranges that represent a mosaic of different landscapes of the West region of Cameroon as described by Temgoua (2011), notably mountainous relief, plateaus and plains: Mbo plain (< 750 m a.s.l.), Tika plain (750–1,000 m a.s.l.), Noun plain (1,000–1,250 m a.s.l.), Bamoun plateau (1,250–1,500 m a.s.l.), Bamileke plateau (1,500–1,750 m a.s.l.) and the isolated volcanic massif (> 1,750 m a.s.l.).

During each sampling night, mist nets (12 m × 2.5 m; mesh, 40 mm) were deployed at particular sites based on prior knowledge of bat activity (over slow-flowing streams, cultivated farms, clearings, cave openings and tree hollows). Mist nets were deployed at each site between 6 pm to 12 midnight and checked every 15 min. For each bat captured, morphometric measurements were taken using a Vernier caliper (Ecotone-Poland 150/0.1 mm), weight was recorded using a Pesola spring balance (Ecotone-Poland Light Line 200g/0.2), the sex, reproductive conditions and age class were also noted. Morphological measurements from each captured bat were used for the identification of each species using the keys in Rosevear (1965), Hayman and Hill (1971), Patterson and Webala (2012) and Happold & Happold (2013). Bats were released after identification and species that could not be identified in the field were kept as voucher, and preserved in 70% alcohol and deposited at the Laboratory of Biological Sciences of the University of Maroua-Cameroon. The geographical positions of each site sampled were recorded using a hand-held GPS (Garmin eTrex).

### Data Analysis

In order to test the relationship between abundance and species richness, the Generalized Linear Mixed-effects Models (GLMMS) was used to discern the potential effects of some altitudinal variables. We performed a Kruskal-Wallis ANOVA followed by HSD tests (at 95 % family-wise confidence level) in order to determine



**Fig. 1.** Map of Cameroon showing the West Region, and sites sampled for bats from November 2016 to November 2018.

if species richness, abundance, diversity and equitability differed among elevation ranges. The software Estimate S 9.0 (Colwell 2013) was used to calculate the number of species ( $X$ ) using the averages of Chao 1 (mean), ACE (mean), Jack 1 (mean) and Bootstrap (mean) and to generate a species accumulation curve. The sampling efficiency was calculated based on the formula below:

$$\text{Sampling efficiency} = \frac{\text{observed number of species}}{\text{estimated number of species}} \times 100$$

Cluster analysis was performed for all altitudinal ranges to test the degree of similarity between them and to test if bat communities represent different assemblages. The Sorenson index (Sorenson 1948), was calculated and used in cluster analysis following the UPGMA (Unweighted Pair-Group) method using the arithmetic aver-

age (Magurran & McGill 2011). Sorenson/Bray-Curtis similarity dendrogram was then plotted using packages of R software version 3.4.1 (R Core Team 2017).

## RESULTS

### Species richness and sampling success

During 95 sampling nights, we recorded a total of 442 bats, comprising 25 species, 16 genera and six families. The family Pteropodidae had 7 species, followed by Hipposideridae with 6 species, Vespertilionidae with 5, Rhinolophidae with 4, Molossidae with 2, and Nycteridae with 1 species (Table 1). The species with the highest capture frequency in the region was *Myonycteris angolensis* (Bocage, 1898) (18.1% of all captures), followed

**Table 1.** Individuals per species captured along an elevational gradient in the West region of Cameroon sampled from December 2016 to November 2018

TAXON	Range I <750m	Range II 750–1,000m	Range III 1,000–1,250m	Range IV 1,250–1,500m	Range V 1,500–1,750m	Range VI >1,750m	Total
<b>Pteropodidae</b>							
<i>Micropteropus pusillus</i>	2	18	20	12	7	1	60
<i>Eidolon helvum</i>	1	1	18	41	0	0	61
<i>Myonycteris torquata</i>	0	1	2	6	2	0	11
<i>Myonycteris angolensis</i>	0	0	8	42	30	0	80
<i>Rousettus aegyptiacus</i>	13	0	1	0	0	0	14
<i>Hypsognathus monstrosus</i>	0	0	1	0	0	0	1
<i>Epomops franqueti</i>	0	1	0	0	0	0	1
<b>Vespertilionidae</b>							
<i>Pipistrellus nanulus</i>	0	0	0	0	0	1	1
<i>Pipistrellus grandidieri</i>	2	0	0	0	0	0	2
<i>Neoromicia nana</i>	1	0	2	0	4	0	7
<i>Scotoecus hirundo</i>	1	0	0	0	0	0	1
<i>Neoromicia tenuipinnis</i>	0	0	4	0	0	0	4
<b>Hipposideridae</b>							
<i>Hipposideros abae</i>	1	0	0	0	0	0	1
<i>Hipposideros beatus</i>	2	0	0	0	0	0	2
<i>Doryrhina cyclops</i>	11	0	3	0	0	0	14
<i>Hipposideros ruber</i>	15	26	0	0	0	0	41
<i>Hipposideros caffer</i>	0	0	1	0	0	0	1
<i>Hipposideros fuliginosus</i>	25	0	4	0	0	0	29
<b>Molossidae</b>							
<i>Chaerephon pumilus</i>	0	0	29	0	4	0	33
<i>Mops nanulus</i>	0	0	15	0	0	0	15
<b>Rhinolophidae</b>							
<i>Rhinolophus landeri</i>	0	28	0	8	0	1	37
<i>Rhinolophus clivosus</i>	0	1	0	0	0	0	1
<i>Rhinolophus simulator</i>	0	22	0	0	0	0	22
<i>Rhinolophus alcyone</i>	2	0	0	0	0	0	2
<b>Nycteridae</b>							
<i>Nycteris arge</i>	0	1	0	0	0	0	1
Species abundance	76	99	108	109	47	3	442
Species diversity	12	9	13	5	5	3	25
Shannon diversity( H' )	2.8	2.3	3.0	1.9	1.7	1.6	3.7

by *Eidolon helvum* (Kerr, 1792) (13.8%) and *Micropteropus pusillus* (Peters, 1868) (13.6%) (Table 1).

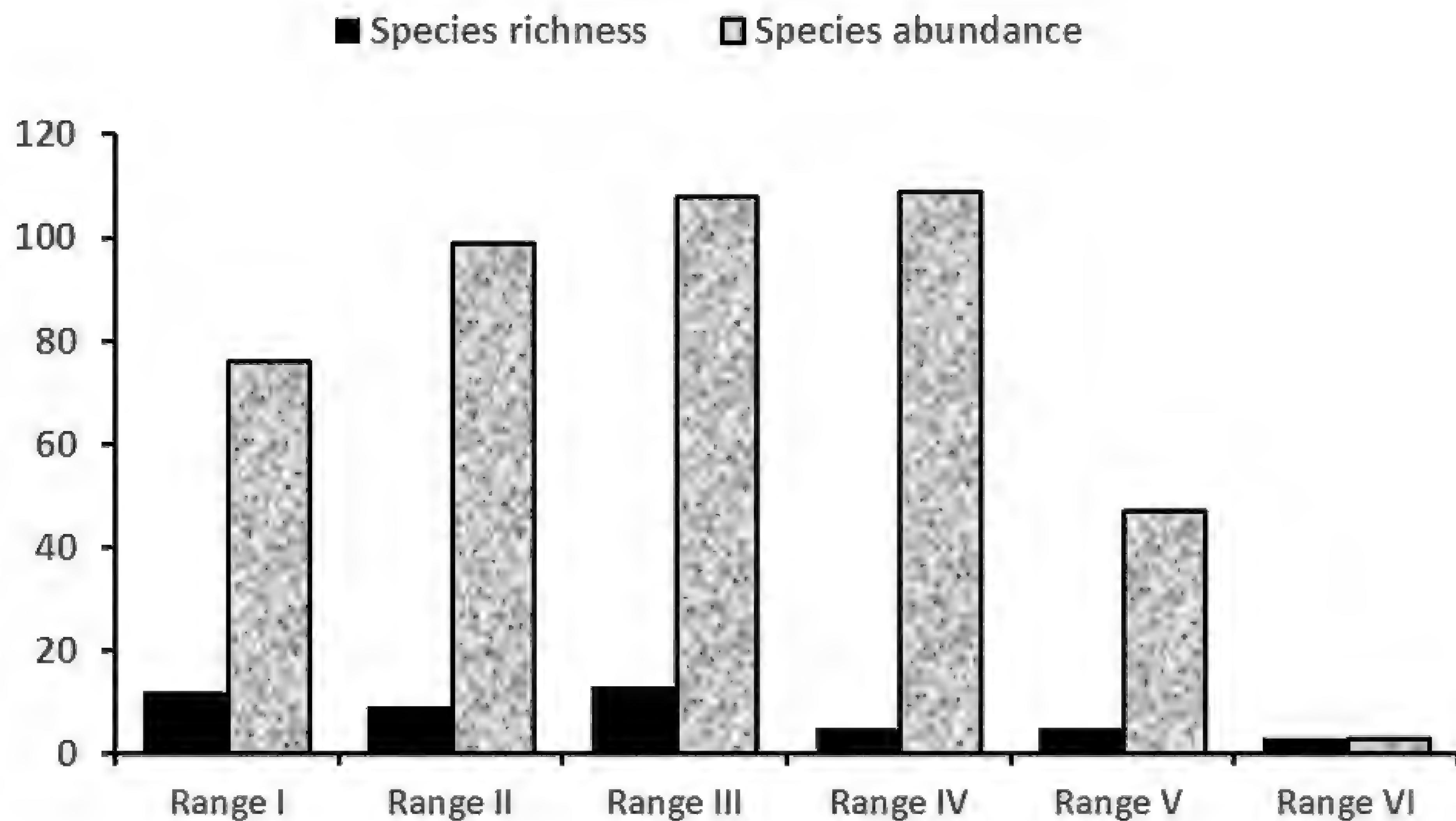
Species richness was highest in elevational range III (1,000–1,250 m a.s.l.) (13 species) and its bat fauna was represented by Pteropodidae, Vespertilionidae, Hipposideridae and Molossidae (Table 1). *Chaerephon pumilus* (Cretzschmar, 1826) (n = 29), was the most abundant species captured at this elevational range, followed by *Micropteropus pusillus* (n = 20) and *Mops* (*Xiphonycteris*) *nanulus* J. A. Allen, 1917 (n = 15). In elevation range I (< 750 m a.s.l.), we recorded a total of 12 spe-

cies, represented by Pteropodidae, Vespertilionidae, Hipposideridae and Rhinolophidae. *Hipposideros fuliginosus* (Temminck, 1853) was the most abundant (n = 25), followed by *Hipposideros ruber* (Noack, 1893) (n = 15) and then *Rousettus aegyptiacus* (E. Geoffroy, 1810) (n = 13). In elevational range II (750–1,000 m a.s.l.), we recorded 9 species, belonging to Pteropodidae, Hipposideridae, Rhinolophidae and Nycteridae. *Rhinolophus landeri* Martin, 1838 was the most frequently captured bat (n = 28), followed by *Hipposideros ruber* (n = 26) and then *Rhinolophus simulator* K. Andersen, 1904 (n =

22). At elevational range IV (1,250–1,500 m) we recorded 5 species, represented by the families Pteropodidae and Rhinolophidae. Elevation range VI (> 1,750 m a.s.l.) was the least diverse, with 3 species (*Pipistrellus nanulus*, *Rhinolophus landeri* and *Micropteropus pusillus*). There was considerable difference in species diversity and abundance across the different elevational ranges (Table 1; Fig. 2).

### Species similarity along the elevational gradient

The Sorenson/Bray Curtis similarity test revealed that the bat community structure of the six different elevational ranges were similar ( $r$ -value = 0.8395) (Fig. 4). Range I (< 750 m) and range II (750–1,000 m) form a similar cluster, and range III (1,000–1250 m) and range IV (1,250–1,500 m) also form a similar cluster, indicating



**Fig. 2.** Species diversity and abundance recorded at each elevational range in the West Region of Cameroon from November 2016 to November 2018.

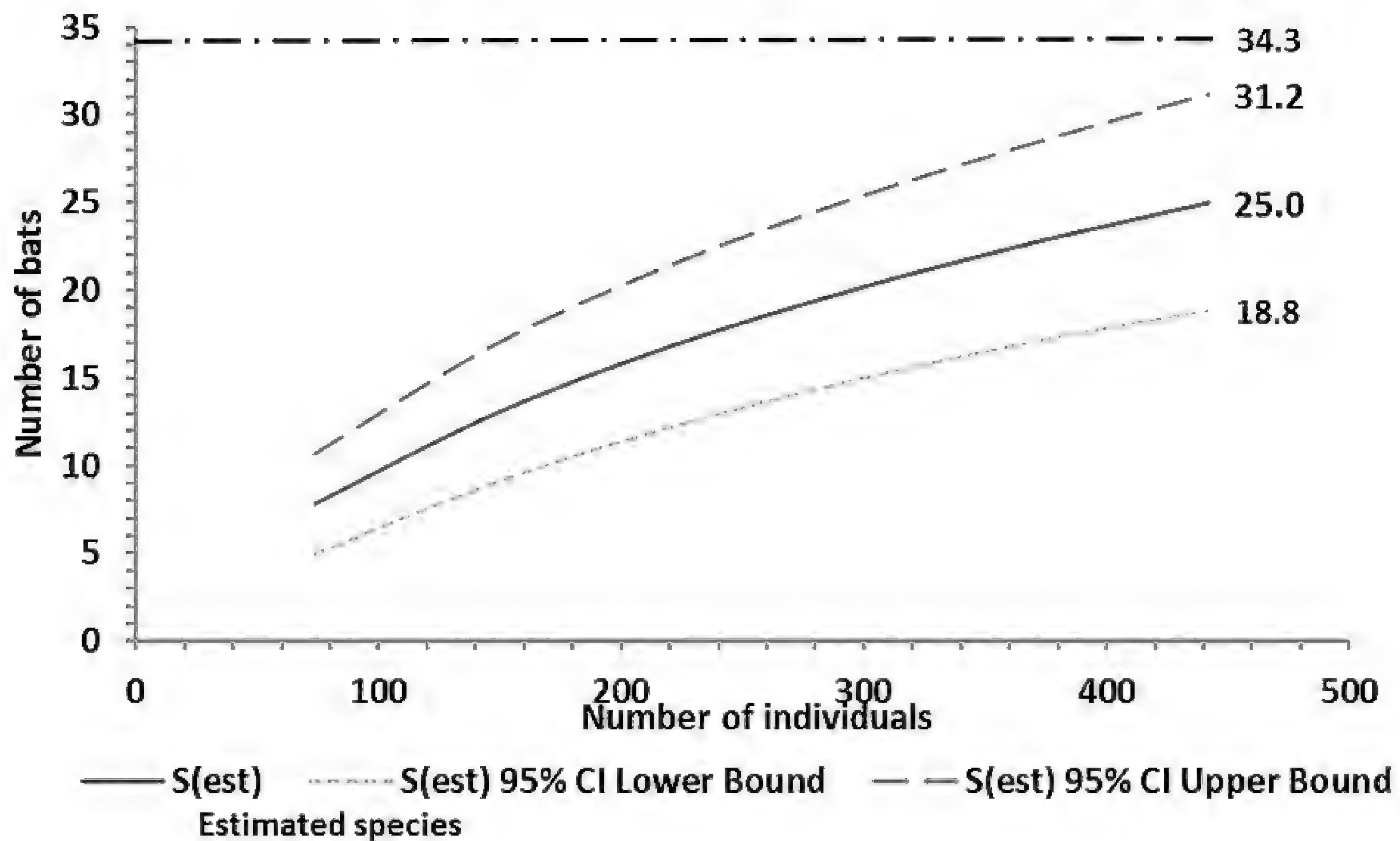
### Species richness and diversity along the elevational gradient

Estimated species richness using Chao 1 (mean), ACE (mean), Jack 1 (mean) and Bootstrap (mean) is 31.9; 38.5; 36.7; and 30.2 species respectively. The average ( $\bar{x}$ ) of these four estimators is 34.3 species. The species accumulation curve in the study area did not reach an asymptote (Fig. 3), indicating that our sampling was incomplete. The sample efficiency was 72.8 %, which indicates that additional survey work is needed. The results of the non-parametric one-way Kruskal-Wallis ANOVA showed that mean species abundance of the six altitudinal ranges did not differ statistically significantly at  $p < 0.05$  level ( $\chi^2 = 2.8651$ ,  $df = 5$ ,  $P = 0.7208$ ). There was also no statistically significant difference in species richness between elevational ranges ( $\chi^2 = 0.38798$ ,  $df = 3$ ,  $p = 0.9427$ ).

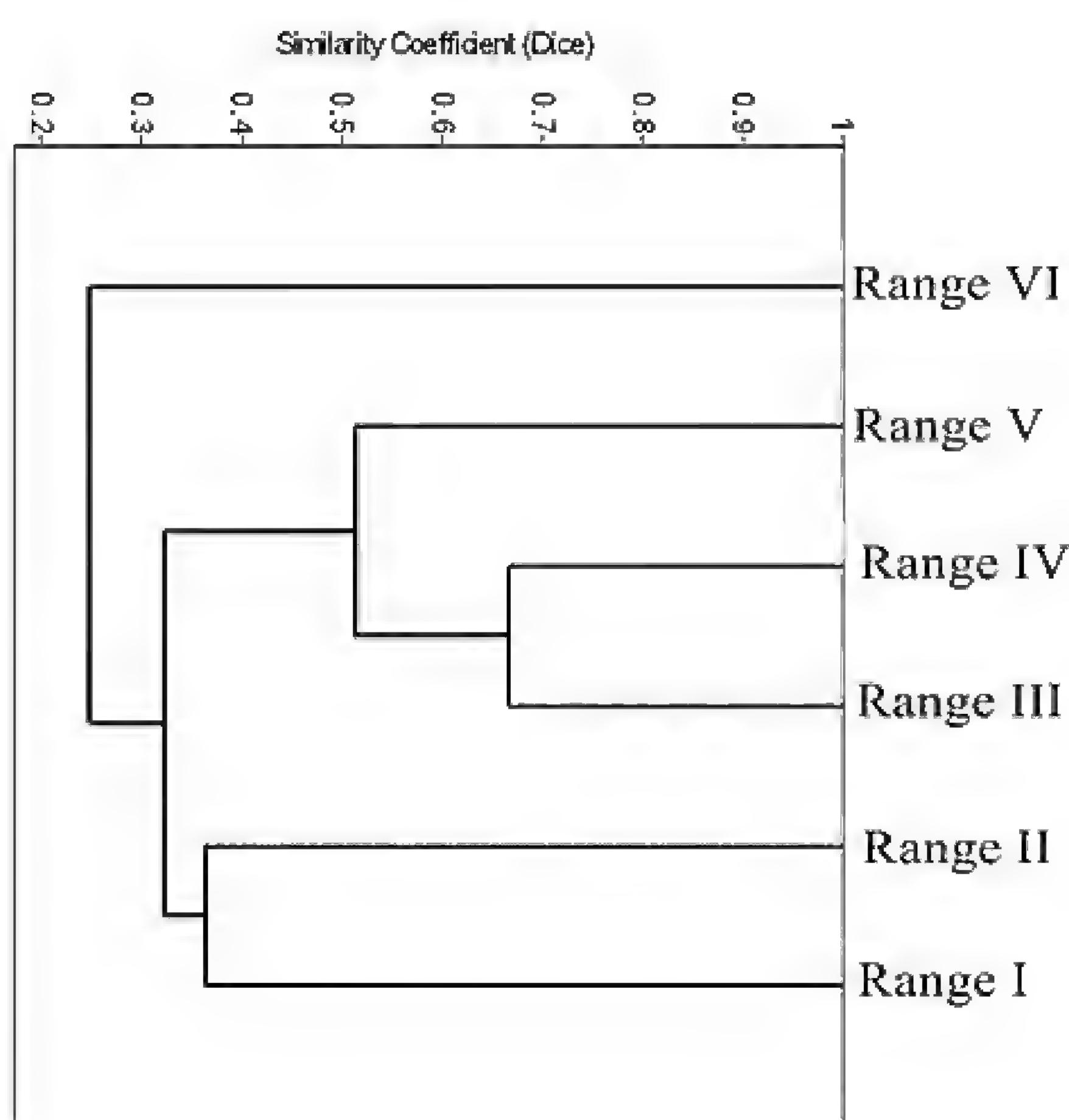
similarity in bat community structure. The dendrogram also indicates that bat community structure of elevational range V (1,500–1,750 m a.s.l.) and elevational range IV was quite different from the cluster formed by range I (< 750 m) and range II (750–1,000 m) and that formed by range III (1,000–1250 m) and range IV (1,250–1,500 m) (Fig. 4).

### Altitudinal species richness and abundance relationship

The results of the Generalized Linear Mixed-effects Models (GLMMS) showed that there is variation in bat species richness and abundance along the altitudinal gradient. There is also a negative relationship between bat species richness and altitude. Indeed, it summarizes the output of the binary logistic model used to discern the potential effects of some altitudinal range on the observed



**Fig. 3.** Species accumulation curve of bats captured in the West Region of Cameroon from November 2016 to November 2018. Horizontal line (34.3) = average of four species richness estimators.



**Fig. 4.** Sorenson/Bray – Curtis similarity test Dendrogram for altitudinal ranges of bats captured in the West region of Cameroon from November 2016 to November 2018.

total species richness. The model showed no statistically significant difference in species richness at  $p < 0.05$  level among the six elevational ranges (estimate =  $0.757 \pm 0.357$ ,  $t = 2.118$ ,  $p = 0.0341$ ). The smallest  $t$ -value found between the elevational ranges I and III, showed no statistically significant difference in species composition. The  $t$ -values of comparisons of the species composition between the groups, IV, V and VI showed the highest difference.

Abundance was positively correlated with elevational ranges I, IV and VI respectively (estimate =  $1.216 \pm 0.195$ ,  $z = 6.227$ ,  $P < 0.001$ ), (estimate =  $0.824 \pm 0.360$ ,  $z = 2.290$ ,  $P = 0.02200$ ) and (estimate =  $-1.620 \pm 0.701$ ,  $z = -2.309$ ,  $p = 0.02092$ ). However, the elevational range III and V respectively (estimate =  $-0.17492$ ,  $z = -1.084$ ,  $p = 0.27815$ ), (estimate =  $-0.39744$ ,  $z = -0.992$ ,  $p = 0.32107$ ) were negatively correlated with altitude.

## DISCUSSION

Our data show that bat species richness in the western region of Cameroon shows a low-plateau with a mid-elevational peak at elevational range III (1,000–1,250 m a.s.l.), after which species richness declines (Table 1). A low-plateau with a mid-elevational peak was also observed for bats at Mount Mulanje, Malawi (Curran et al.

2012). A number of hypotheses have been proposed to explain the decrease in species richness with altitude among which the most frequently cited explanations include the mid-domain effect (MDE) (Rahbek 1997) and environmental factors such as climatic variables, productivity and habitat heterogeneity (Nogués-Bravo et al. 2008; Sanders & Rahbek 2012). Indeed, Peters et al. (2016) stressed the importance of temperature as the main predictor of species diversity in both plant and animal communities. Furthermore, Peters et al. (2019) specified that variation in species diversity in tropical mountains is mostly driven by the interaction of both climate and human land use changes. The MDE on the other hand suggest that if species ranges are randomly shuffled within a bounded geographical domain free of environmental gradients, ranges overlap increasingly toward the center of the domain, creating a “mid-domain” peak of species richness (Colwell & Hurtt 1994; Colwell & Lees 2000). According to McCain (2009) water availability is higher at lower elevations. More open water leads to an increase in activity of insects and subsequently to an increase in activity of insectivorous bats (Korine & Pinshow 2004). Furthermore, MacArthur & MacArthur (1961) argued that lower elevations may also possess greater structural complexity in vegetation that provide more resources and hence support a larger number of species. However, differences in species richness pattern observed along the elevational gradient may also be explained by the interaction between climatic factors, vegetation structure and anthropic land use. Also, more species were captured at lower and intermediate elevations than at the highest elevation, this can be partly explained by the fact that relatively fewer sites were sampled at higher elevations.

Twenty-five bat species were recorded at different elevational ranges. *Myonycteris angolensis* was the most abundant bat captured. This forest species is common in Cameroon, and was previously recorded in Buea (Matschie 1891), Bibundi, Bonge, Ndiang, (Sjöstedt 1897a, b), Bimbia, Tombel (Eisentraut 1941), Eseka (Haiduk et al. 1981), Ngaoundere (Müller et al. 1981), Dja Reserve (Bakwo Fils 2009), and Mpem and Djim National Park (Atagana et al. 2018). The abundance of this species at mid-elevations may be due to the existence of numerous natural and manmade structures that provide day roosts. Meredith Happold pointed out that *Myonycteris angolensis* is apparently common in some localized areas where fruiting trees and caves or cave-like day roosts are present (Happold 2013a – as *Lissonycteris angolensis*). Indeed, ACR (2018) established that *Myonycteris angolensis* is widely distributed at elevations ranging from sea level to 4000 m a.s.l. in Africa. *Micropteropus pusillus* was recorded in all ranges and its distribution is not homogeneous across the elevation gradient. According to Atagana et al. (2018), *Micropteropus pusillus* is widespread in Cameroon, and inhabits forest, savannah, plantation and ecotone areas. Eisentraut (1973)

also recorded *Micropteropus pusillus* at elevations up to 1,800 m at Mount Manengouba. *Eidolon helvum*, *Rousettus aegyptiacus* and *Myonycteris torquata* were recorded at low and mid-elevations. *Eidolon helvum* was recorded at elevations between 750 m and 1,500 m. Curran et al. (2012) on Mount Mulanje in Malawi recorded *E. helvum* only at mid-elevation (1,220–1,320 m). Indeed, this species also preferred disturbed habitats and forms large colonies around human habitations. *Rousettus aegyptiacus* was only recorded at elevation below 1,250 m and was conspicuously absent at higher altitudes. This result contrast with that obtained in West Africa by Verschuren (1976) and Denys et al. (2013) who reveal the abundance of *Rousettus aegyptiacus* at high elevations. Kwiecinski & Griffiths (1999) showed that the abundance of *Rousettus aegyptiacus* at high elevation may be due to the existence of numerous caves that provide day roost. In Cameroon, this forest species was previously recorded in both primary and secondary forest by Sanborn (1936), Sanderson (1940), Maisel et al. (2001), Bakwo Fils (2009) and Atagana et al. (2018). *Epomops franqueti* (Tomes, 1860) was recorded at mid-altitudes. These findings are similar to those obtained by Eisentraut (1973) who recorded *Epomops franqueti* at an altitude below 1,000 m on Mount Cameroon. Our study recorded only a single individual of *Hypsognathus monstrosus* H. Allen, 1861 at mid-altitudes. According to Bergmans (1989), *Hypsognathus monstrosus* is common in the rainforest, and its abundance is determined by the availability of ripe fruits. This species inhabits areas below 1,800 m and was not recorded beyond this elevation (Happold 2013b).

This study revealed that vespertilionids were recorded in all altitudinal ranges. *Pipistrellus nanulus* Thomas, 1904 and *Neoromicia tenuipinnis* (Peters, 1872) were recorded at high and mid-altitudes respectively. *Pipistrellus* cf. *grandidieri* (Dobson, 1876) and *Scotoecus hirundo* (de Winton, 1899) were recorded at low altitudes. However, *Neoromicia nana* was recorded at both low and mid-altitudes. According to Soriano (2000), some species of vespertilionids may be better adapted to higher altitudes with colder climate despite their size. Bat species of the family Molossidae were captured at mid-altitudes around man-made structures that provide day roost. These findings corroborate those of McWilliam (1989) and Esbérard (2003) who suggested that there is a high probability of sampling molossids in front of their exit or near possible roosts in roof linings of human residences. Bats of the family Hipposideridae, Rhinolophidae and Nycteridae have a higher richness and abundance in both low and mid-altitudes. Our result is consistent with the findings of Curran et al. (2012) who previously captured a higher amount of bat species of the families Hipposideridae, Rhinolophidae and Nycteridae between 630 and 1,030 m altitude on Mount Mulanje in Malawi. Rosevear (1965), Schober & Grimmberger (1997), Georgiakakis (2010) and Happold & Happold (2013) suggested that

the distribution of some species like *Hipposideros ruber*, *H. fuliginosus*, *Rhinolophus landeri* and *R. simulator* are most consistently associated with day roosts and food availability. Our data reveals the presence of a single individual of *Rhinolophus landeri* Martin, 1838 at high altitude in a cultivated area. At Mount Cameroon, Rosevear (1965) recorded *R. landeri* in montane vegetation at 1,400 m, while Largen et al. (1974) captured it in Ethiopia from 515 to 1,800 m and found no marked altitudinal preference. We recorded only a single individual each of *Hipposideros abae* J. A. Allen, 1917, *H. caffer* (Sundevall, 1846), *Rhinolophus clivosus* Cretzschmar, 1828, and *Nycteris arge* Thomas, 1903 in our study, probably indicating the rarity of these species.

This study has allowed us to obtain data on the distribution of bat species with respect to the landscape of the western region of Cameroon. However, we observed that insectivorous bats are more diversified (18 species) but less abundant (214 individuals) compared to frugivorous bats that are less diverse (seven species), but more abundant (228 individuals). This may be related to a bias in the capture method that involved the use of understory mist nets that are known to be efficient at capturing sub-canopy frugivorous bats (Fleming 1982). Additionally, it is well established that mist nets have good success only in open environments (streams, cultivated farms, clearings) (Martins et al. 2015). As pointed out by Kaňuch & Krištín (2006), Rhinolophidae, Hipposideridae and some Vespertilionidae can easily avoid mist nets because of their efficient echolocation calls. Therefore, for more exhaustive studies we recommend the use of harp traps, echolocation recording and canopy netting to supplement standard mist-netting. Our sampling efficiency was estimated as 72.8%, confirming that additional surveys might significantly improve our chances of recording species new to the area. The similarity found between the elevational ranges IV and V, and between elevation ranges III and IV, V, is probably be related to the higher turnover of bat species between these elevations (Martins et al. 2015). According to Lomolino (2001), biotic turnover varies along the elevation gradient and depends directly on the richness of the overlapping community.

In conclusion, our study provides baseline data on the altitudinal ranges of bats in the western region of Cameroon. Species richness and abundance was higher at low and mid-elevations but lower at higher altitudes. This pattern of diversity is probably driven by differences in ecological heterogeneity among the different elevational ranges that provide suitable habitats for a number of species. However, further research may be required to evaluate the impact of different habitats types on populations of bat species in the western region of Cameroon.

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## Research article

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# Four new species of the *Hylomyscus anselli* group (Mammalia: Rodentia: Muridae) from the Democratic Republic of Congo and Tanzania

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**Abstract.** As in many other small mammal groups from the Afrotropics, the number of species recognized within the genus *Hylomyscus* has increased considerably over the past dozen years. The last comprehensive review (2005) of the genus recognized eight species. Since that time, nine additional species have been elevated from synonymy (n = 4) or described as new (n = 5). Here we describe four additional new species supported by morphological and molecular evidence, all collected by the late William Stanley. Two of the new taxa are sympatric and come from the poorly known left bank (direction source to mouth) of the Congo River. One of these (*Hylomyscus pygmaeus* sp. nov.) is easily recognized, as it is tiny and significantly smaller than any known species of the genus; the second new species (*Hylomyscus thornesmithae* sp. nov.) is also small, and syntopic with the first. The third new species (*Hylomyscus stanleyi* sp. nov.), from the SW corner of Tanzania, is quite large and had been previously included within the hypodigm of *Hylomyscus anselli* following its recognition from within the synonymy of *Hylomyscus denniae*. The fourth species (*Hylomyscus mpungamachagorum* sp. nov.) is from Mahale Mountains National Park, western Tanzania. Our study reveals a much higher species diversity of the genus than previously known, providing insights into additional Afrotropical and Afromontane centers of endemism that require further exploration.

**Key words.** Afrotropics, biodiversity, endemism, Murinae, molecular phylogeny, systematics, alpha-taxonomy, biogeography.

## INTRODUCTION

Members of the genus *Hylomyscus* (wood mice) are widespread in the forests of sub-Saharan Africa, north of the Zambezi River. They are small (15–35 g), with tails longer than head and body and have skulls with a short upper tooth row and narrow zygomatic plates. Their short broad feet suggested to their describer that they were

more arboreal than their relatives in the genus *Praomys* from which they were split (Thomas 1926). In 2005, Musser and Carleton recognized eight species within the genus: *Hylomyscus aeta* (Thomas, 1911); *H. allenii* (Waterhouse, 1838); *H. baeri* Heim de Balsac & Aellen, 1965; *H. carillus* (Thomas, 1904); *H. denniae* (Thomas, 1906); *H. grandis* Eisentraut, 1969; *H. parvus* Brosset et al. 1965; and *H. stella* (Thomas, 1911). Within one year, this

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total increased to 12 (Carleton et al. 2006) with the description of *H. arcimontensis* Carleton & Stanley, 2005; and the elevation from synonymy of *H. anselli* (Bishop, 1979), *H. vulcanorum* (Lönnberg & Gyldenstolpe, 1925), and *H. endorobae* (Heller, 1910). Since that time, one additional species has been elevated from synonymy (*H. simus* Allen & Coolidge, 1930) and four have been described as new: *H. heinrichorum* Carleton et al., 2015, *H. kerbis peterhansi* Demos et al., 2014, *H. pamfi* Nicolas et al., 2010 and *H. walterverheyeni* Nicolas et al., 2008, bringing the total to 17. Carleton et al. (2006), using phenetic characters, proposed six species groups within the genus ('*H. aeta*', '*H. allenii*', '*H. anselli*', '*H. baeri*', '*H. denniae*', and '*H. parvus*' groups). Eight characters were used to define the '*H. anselli*' group, four of which distinguished it from the '*H. denniae*' group with which it had been previously lumped: six mammae (instead of eight), shorter rostrum, shorter incisive foramina and a medium-sized subsquamosal foramen (compared to a tiny or absent subsquamosal foramen). Two species of the '*H. anselli*' group (*H. anselli* and *H. arcimontensis*) and three of the '*H. denniae*' group (*H. denniae*, *H. vulcanorum* and *H. endorobae*) were recognized at that time.

Today, the '*H. denniae*' group is restricted to the montane highlands of the Albertine Rift and the Kenya Highlands whereas the '*H. anselli*' group is distributed from the Kenya Highlands through the Eastern Arc and Southern Highlands of Tanzania (and northernmost Malawi), into the gallery forests of northern Zambia and the highland plateau of Angola. The other four species groups are confined to the tropical regions of the continent. Here we redefine this geographic pattern by describing four new species of the '*H. anselli*' group from a previously undocumented area of the Congo Basin and two isolated forests in western Tanzania.

## MATERIAL AND METHODS

### Specimens, morphology, morphometrics, collecting

Specimens are from the Field Museum of Natural History, Chicago (FMNH), National Museum of Zambia, Livingstone (NMZ) and British Museum Natural History, London (BMNH). Additional Zambian and Angolan records use the initials from their collectors (RS, R Sumbera) or country of origin (ANG, Angola) and are deposited in the Faculty of Sciences, University of South Bohemia (USB) in České Budějovice (carcasses) and in the Institute of Vertebrate Biology (IVB) of the Czech Academy of Sciences, Studenec (tissues and skulls), both in the Czech Republic. Field measurements, in millimeters, include: Total Length (TL), Tail vertebrae (TV), Hind foot length (HF), Ear length (EL), Weight (Wt, in grams); subtraction of TV from TL provides the head-and-body length (HB) unless measured separately in the

field. All external measurements were taken from original field data by respective collectors. Only two specimens of *H. heinrichorum* have weight recorded (both ANG). Length of hind foot includes the claw. Hind foot measurements of *Hylomyscus heinrichorum* from Angola and all *Hylomyscus anselli* from Zambia specified 'su' (sans unguinal). Accordingly, 1.0 mm was added to HF for missing claw length measurements. We employed the following 16 cranial measurements in millimeters (Carleton & Van der Straeten 1997): occipito-nasal length (ONL), condyle-incisive length (CI), greatest zygomatic breadth (ZB), breadth of the braincase measured across the parietal flanges behind the zygomatic arches (BBC), breadth across the occipital condyles (BOC), least interorbital breadth (IOB), length of nasals (LN), breadth of the rostrum (BR), post-palatal length (PPL), length of the bony palate (LBP), length of the incisive foramen (LIF), length of upper diastema (LD), breadth of the zygomatic plate (BZP), length of the auditory bulla, oblique to tooth row (LAB), coronal (rather than alveolar) length of the maxillary toothrow (CLM), and width of the first upper molar (WM1). We define rostral length as length of nasals divided by occipito-nasal length (LN/ONL). In order to estimate relative age and ontogenetic growth, we adopt dental wear stages from Verheyen & Bracke (1966). Specimens were measured and weighed, and either prepared as skins and skeletons or fixed in formalin and later transferred to 70% ethanol, and deposited at FMNH or USB. DRC refers to the Democratic Republic of Congo and TZ refers to Tanzania. An aliquot of tissue was taken from the specimen at the time of capture and preserved in ETOH until it was transferred to cryogenic storage at -180 °C at FMNH.

Principal components analysis (PCA) of 16 log-transformed crano-dental variables based on a variance-covariance matrix was used to assess morphometric variation and visualize the morphometric distinctiveness of named and putative species for an eight species data set (159 specimens: *H. anselli*, *H. arcimontensis*, *H. heinrichorum*, *H. kerbis peterhansi*, *H. sp. nov. 1* (pygmy *Hylomyscus* from DRC), *H. sp. nov. 2* (small *Hylomyscus* from DRC), *H. sp. nov. 3* (Mbizi *Hylomyscus* from Tanzania), *H. sp. nov. 4* (Mahale *Hylomyscus* from Tanzania) and a four species data set (136 specimens: *H. arcimontensis*, *H. kerbis peterhansi*, *H. sp. nov. 3* (Mbizi *Hylomyscus* from Tanzania) and *H. sp. nov. 4* (Mahale *Hylomyscus* from Tanzania). Standard summary statistics were calculated from univariate measurements for 16 crano-dental and six external characters. All statistical analyses were performed using the software PAST (Hammer et al. 2001).

Permission for the collection and export of specimens was provided by the Republic of Tanzania and the Democratic Republic of Congo. Approval for the import of specimens into the USA was provided by the US Fish and Wildlife Service. Relevant documents pertaining to

export and import are housed at FMNH under the following Accession Numbers: Z-20745, Z-20738, Z-19855, Z-19599. All euthanized specimens followed the protocol approved by the American Society of Mammalogists (Sikes et al. 2011). The study was approved by the Field Museum of Natural History Institutional Animal Care and Use Committee (09-3).

### DNA extraction, amplification, and sequencing

Whole genomic DNA was extracted from tissue samples of *H. aeta* (n = 1, Uganda), *H. stella* (n = 1, Uganda), *H. heinrichorum* (n = 4, Angola), *H. sp. nov. 1* (pygmy *Hylomyscus* from DRC, n = 1), *H. sp. nov. 2* (small *Hylomyscus* from DRC, n = 5), *H. sp. nov. 3* (Mbizi *Hylomyscus* from Tanzania, n = 5), *H. sp. nov. 4* (Mahale *Hylomyscus* from Tanzania, n = 3) using the QIAGEN DNeasy Blood and Tissue Kit (Germantown, MD). An additional 34 *Hylomyscus* cytochrome-*b* (*Cytb*) sequences were downloaded from GenBank (Appendix 2). *Mastomys natalensis*, a close relative of *Hylomyscus* (Steppan & Schenk 2017), was chosen as an outgroup using a *Cytb* sequence downloaded from GenBank. In total, sequence data was generated or downloaded from GenBank for 16 of 18 currently recognized species (Mammal Diversity Database 2019) from all six *Hylomyscus* species groups. Frozen tissues and GenBank accessions were unavailable for *H. carillus* (Angola). Specimens were sequenced for *Cytb* using the primers L14723 and H159125 (Lecompte et al. 2002). PCR amplification was performed on 25 µL reactions using the following thermal conditions: an initial denaturation step at 94 °C for 3 min, followed by 38 cycles consisting of 30 s at 94 °C, 30 s at 50 °C, and 1 min at 68 °C, followed by a final extension step of 5 min at 68 °C. Amplified PCR products were purified using ExoSAP-IT (Thermo Scientific, MA, USA). Sequencing was carried out in both directions on an ABI 3100 thermocycler (Applied Biosystems, CA, USA) at the Pritzker Laboratory for Molecular Systematics and Evolution (FMNH). Chromatographs were checked manually and assembled and edited using Geneious 11.1.5 (Biomatters Ltd.). Sequences were aligned for *Cytb* using MUSCLE Alignment within the Geneious platform with default parameters. Sequence data from *Cytb* were translated into amino acids and the alignment was inspected for deletions, insertions, and premature stop codons to exclude possible nuclear pseudogenes.

### Molecular data and phylogenetic analyses

The best supported model of nucleotide substitution for *Cytb* was determined using the BIC on the maximum likelihood topology inferred in jMODELTEST2 v.2.1.6 (Darriba et al. 2012) on CIPRES Science Gateway v.3.3 (Miller et al. 2010). Interspecific uncorrected sequence divergences (*p*-distances) were calculated in MEGA

7.0.26 (Kumar et al. 2016). Maximum likelihood inference of a *Cytb* gene tree was made using the program IQ-TREE v1.6.10 (Nguyen et al. 2015) on the CIPRES portal. We conducted analyses using the best-scoring ML tree search algorithm under the GTR+I+G model with 1,000 bootstrap replicates. Bayesian gene tree analyses were carried out using MRBAYES v.3.2.6 (Ronquist et al. 2012) on the CIPRES portal to infer a *Cytb* gene tree. Two replicates were run to facilitate proper mixing. Four Markov chains with default heating values were conducted for 10,000,000 generations and sampled every 1,000th generation. Stationarity of the MCMC chain was assessed using TRACER v.1.7.1 (Rambaut et al. 2018). The first 2,500 samples were discarded as burn-in and the remaining 7500 samples comprised the posterior probability (PP) distributions. A majority rule consensus tree was generated from the analysis. All newly generated sequences were deposited in GenBank with accession numbers MN857618–MN857637 (Appendix 2). We use these gene tree analyses to test the concordance of species limits inferred using morphological data with clades supported by genetic data, estimate support for monophyly of recognized and putative species, and assess phylogenetic relationships among them.

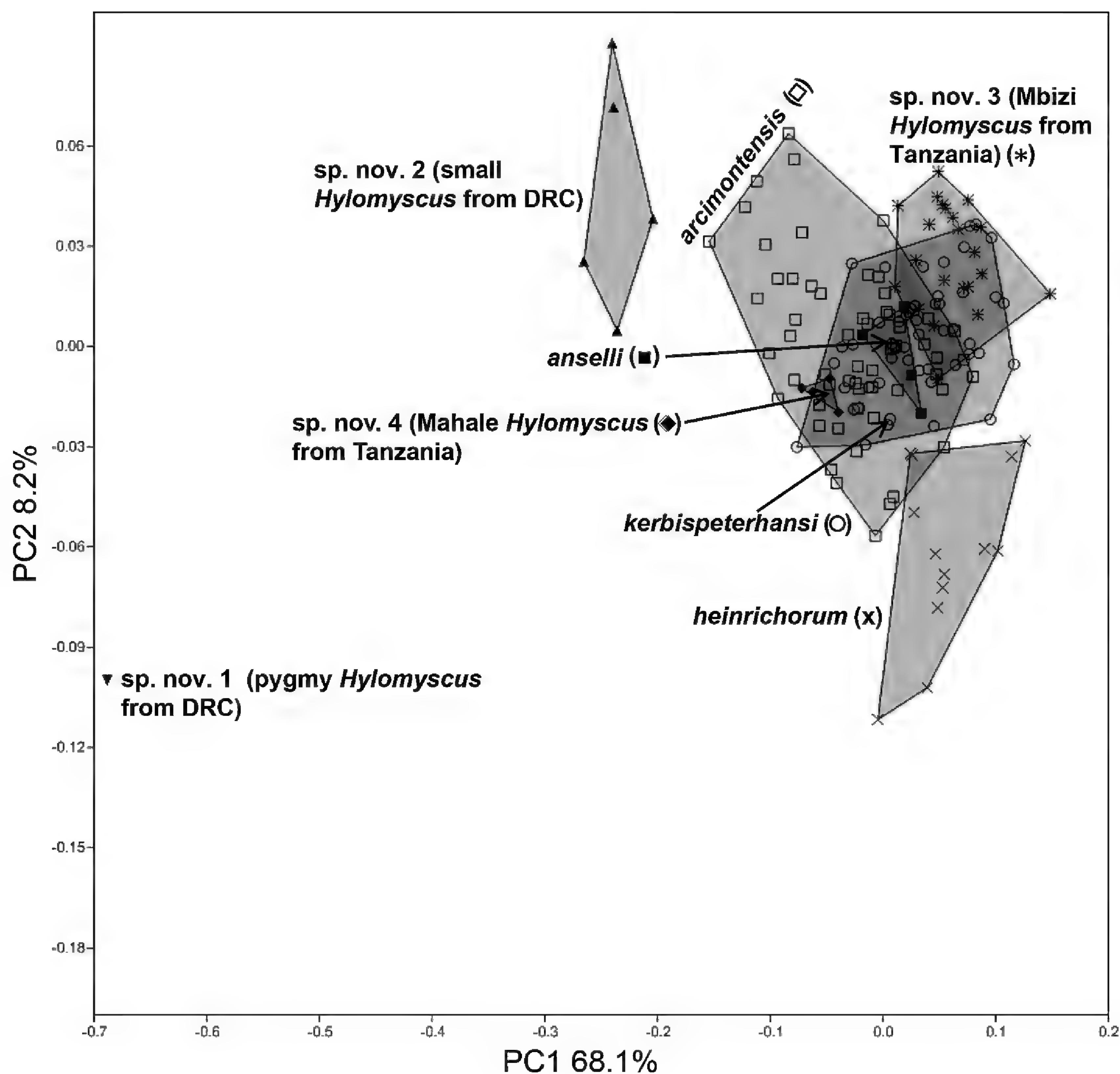
## RESULTS

The specimens from the Congo basin (DRC) were clearly undescribed members of the *H. anselli* clade based on their morphological characters: short feet, teat formula (2+4 for *Hylomyscus* sp. nov., small *Hylomyscus*), short incisive foramen, thin stapedial strap and medium-size subsquamosal foramen. Their small size confirmed their unique status within the *H. anselli* clade. Subsequent genetic analyses were instrumental in revealing the existence of the two cryptic Tanzanian species.

### Morphometrics

Two principal component analyses were performed on 16 log-transformed cranio-dental variables. The first included all eight putative species of the *H. anselli* group (Fig. 1); the second (Fig. 2) included those four species found east of the Albertine Rift Valley (Fig. 3). In the eight species PCA (Fig. 1), the small-sized Congo Basin taxa (*H. sp. nov. 1*, pygmy *Hylomyscus* from DRC and *H. sp. nov. 2* (small *Hylomyscus* from DRC) are distinguished from all other species in the *H. anselli* group along the first axis and will not be discussed further. The Angolan taxon (*H. heinrichorum*) is distinguished along the second axis from the Zambian taxon (*H. anselli*).

In the four species PCA (Fig. 2), *H. kerbispetterhansi* and *Hylomyscus* sp. nov. 3 (Mbizi *Hylomyscus* from Tanzania) are readily distinguished along the second axis. *Hylomyscus* sp. nov. 3 (Mbizi *Hylomyscus* from



**Fig. 1.** Principle component analysis of cranial measurements of all eight members of the *Hylomyscus anselli* group.

Tanzania) has only modest overlap along the 1<sup>st</sup> and 2<sup>nd</sup> axes with *H. arcimontensis*, but *H. kerbispeterhansi* and *H. arcimontensis* show overlap along both principle components. *Hylomyscus* sp. nov. 4 (Mahale *Hylomyscus* from Tanzania) does overlap with *H. arcimontensis* in PCA multivariate space (Fig. 2) but they are allopatric and occupy different biogeographic regions: *Hylomyscus arcimontensis* is confined to the Eastern Arc montane archipelago while *Hylomyscus* sp. nov. 4 (Mahale *Hylomyscus* from Tanzania) is known only from the Albertine Rift; see Fig. 3).

In the eight species PCA, the first two principle components accounted for 76% of the cumulative variance (Table 1). The major contributing variables on PC1 were all correlated with the length of the rostrum: length of incisive foramen (LIF), length of nasals (LN) and length of

diastema (LD). On PC2 the major contributing variables were breadth of zygomatic plate (BZP), length of nasals (LN), and length of bony palate (LBP). In the four species PCA the first two principle components accounted for 64% of the cumulative variance (Table 2). The major contributing variables on PC1 were identical to PC1 on the eight species PCA: length of diastema (LD), length of incisive foramen (LIF) and length of nasals (LN). On PC2, the most important variables were breadth of zygomatic plate (BZP), length of bony palate (LBP) and crown length of molars (CLM).

#### Genetic analyses

The mtDNA gene tree (Fig. 4) supports the monophyly of the *Hylomyscus anselli* group minus *H. sp. nov. 1*

**Table 1.** PCA loadings from all eight members of the *Hylomyscus anselli* group.

Variable	Correlations	
	PC1	PC2
ONL	0.240	0.106
CI	0.253	0.095
ZB	0.192	0.102
BBC	0.138	0.040
BOC	0.143	-0.096
IO	0.152	-0.094
LN	0.359	0.427
BR	0.209	0.183
PPL	0.212	0.089
LBP	0.208	0.359
LIF	0.381	-0.220
LD	0.302	0.196
BZP	0.281	-0.636
LAB	0.234	-0.015
CLM	0.277	-0.208
WM1	0.267	-0.251
Cumulative % variance	68.1	76.3
Eigenvalue	0.0081	0.0010

**Table 2.** PCA loadings from four members of the *Hylomyscus anselli* group found east of the Albertine Rift.

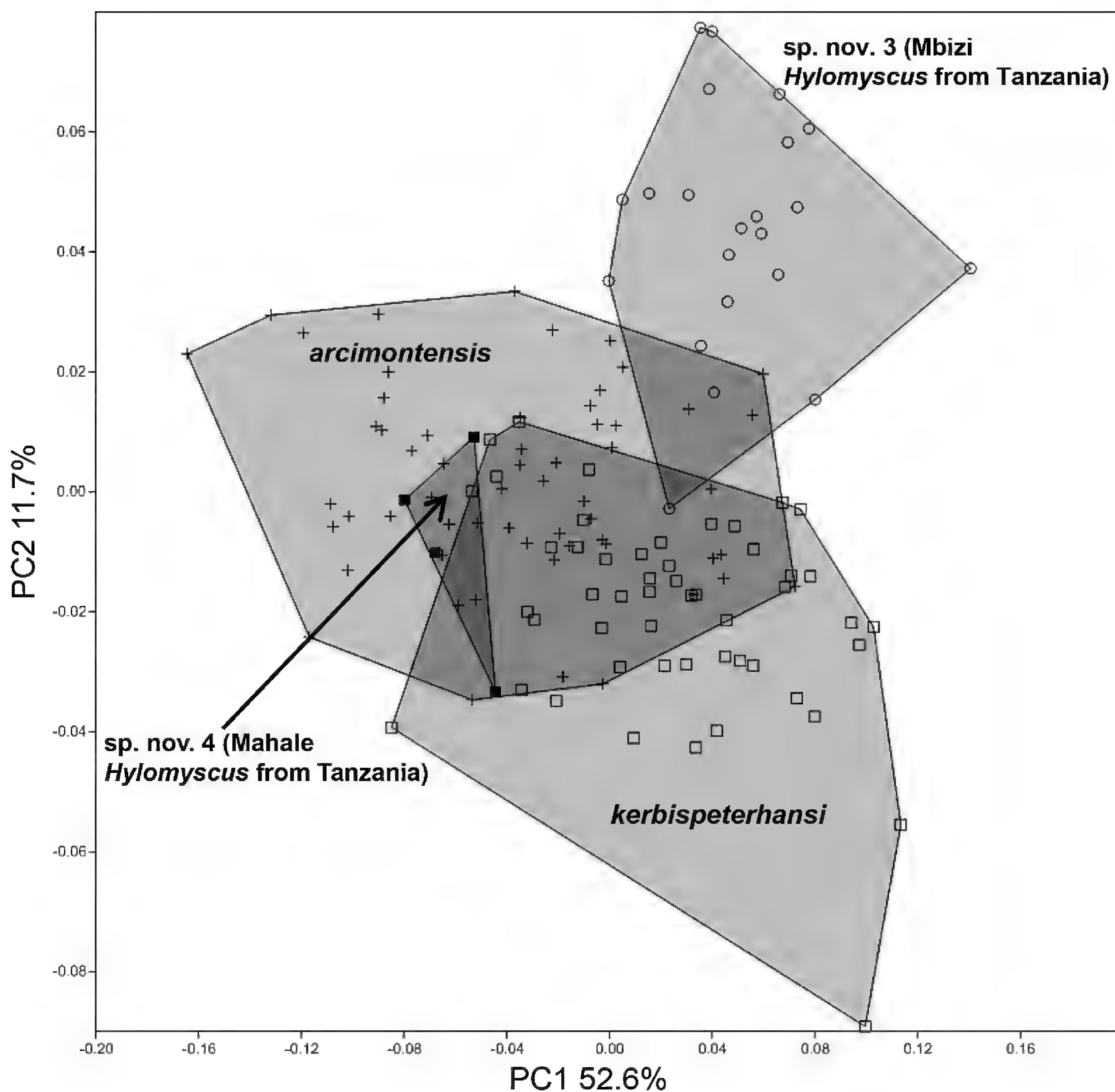
Variable	Correlations	
	PC1	PC2
ONL	0.231	0.102
CI	0.267	-0.027
ZB	0.211	0.037
BBC	0.155	0.107
BOC	0.146	-0.006
IO	0.160	0.216
LN	0.314	0.327
BR	0.278	-0.131
PPL	0.260	0.044
LBP	0.208	0.392
LIF	0.325	-0.229
LD	0.340	-0.222
BZP	0.280	-0.546
LAB	0.298	-0.209
CLM	0.215	0.375
WM1	0.197	0.258
Cumulative % variance	52.6	64.3
Eigenvalue	0.0034	0.0008

(pygmy *Hylomyscus* from DRC; bootstrap [BS] = 99, posterior probability [PP] = 1.0). The new species, *Hylomyscus* sp. nov. 1 (pygmy *Hylomyscus* from DRC) from the western Congo basin, is highly divergent from other *H. anselli* group species (10.9–12.1% *Cytb* *p*-distance), and moderately well supported as sister to all other *H. anselli* group members (BS = 82, PP = 0.96). The new species *Hylomyscus* sp. nov. 2 (small *Hylomyscus* from DRC), *H.* sp. nov. 3 (Mbizi *Hylomyscus* from Tanzania) and *H.* sp. nov. 4 (Mahale *Hylomyscus* from Tanzania) are well supported clades (bootstrap = 100, posterior probability = 1.0). Carleton and Stanley (2005) and Carleton et al. (2006, 2015) assigned specimens from the Mbizi Mountains, Tanzania, to *H. anselli* based on similar phenetics and geographical proximity. However, our PCA (Fig. 1) shows little overlap between *H. anselli* and *H.* sp. nov. 3 (Mbizi *Hylomyscus* from Tanzania), although we had access to only four members of *H. anselli*. Further, our newly available genetic data indicate that this assignment was incorrect. The Mbizi *Hylomyscus* population is now assigned to distantly related *H.* sp. nov. 3 (Mbizi *Hylomyscus* from Tanzania, Fig. 4), illustrating how ‘cryptic’ these species are. *H. anselli* and *H.* sp. nov. 3 (Mbizi *Hylomyscus* from Tanzania) are not even sister; the genetic distance between them is 8.2% at *Cytb* (Table 3). *Hylomyscus* sp. nov. 3 (Mbizi *Hylomyscus* from Tanzania) + *H.* sp. nov. 4 (Mahale *Hylomyscus*

from Tanzania) + *H. kerbispeterhansi* form a distinct and highly supported East African clade (BS = 82, PP = 0.96) distributed in montane habitats in Kenya and Tanzania. These species exhibit allopatric distributions within the *H. anselli* group, although *H. kerbispeterhansi* is sympatric with *H. endorobae* (*H. denniae* group) on the Mau Escarpment in west-central Kenya (see Demos et al. 2014a, b; 2015). The relationship of *H. arcimontensis* from Tanzania is weakly supported (BS = 79, PP = 0.87) as sister to the aforementioned three East African *H. anselli* group species. *Hylomyscus* sp. nov. 2 (small *Hylomyscus* from DRC), also from the western Congo Basin, is strongly supported (BS = 92, PP = 1.0) as sister to the geographically distant and disjunct East African *H. anselli* group clade. *Hylomyscus anselli*, now restricted to Zambia on the basis of data from this study, is strongly supported (BS = 100, PP = 1.0) as sister to *H. heinrichorum* from Angola, and this clade (*H. anselli* + *H. heinrichorum*) is strongly supported (BS = 99, PP = 1.0) as sister to a clade that includes representatives of the *H. anselli* group from both East Africa and the western Congo Basin.

### New taxa

All four new species (*Hylomyscus* sp. nov. 1, sp. nov. 2, sp. nov. 3, sp. nov. 4) described here nest within the *H. anselli* group. Of the characters suggested to define the *H.*



**Fig. 2.** Principle component analysis of cranial measurements of the four members of the *Hylomyscus anselli* group found east of the Albertine Rift.

*anselli* group (Carleton et al. 2006), the most reliable include abbreviated incisive foramina, the absence of pectoral mammae (total mammae = 2+4) and the thin, elongate hamular process providing for enlarged sub-squamosal foramina (discussed and illustrated in Carleton & Stanley 2005: fig. 6). These characters distinguish the *H. anselli* group from the *H. denniae* group, the only two groups with montane representatives to the east and south of the Congo Basin. All of the four new taxa described below possess these three characters; however, in *Hylomyscus pygmaeus* sp. nov. (pygmy *Hylomyscus* from DRC), teats are not visible, as the specimen is young.

***Hylomyscus pygmaeus* sp. nov.** Kerbis Peterhans, Hutterer & Demos

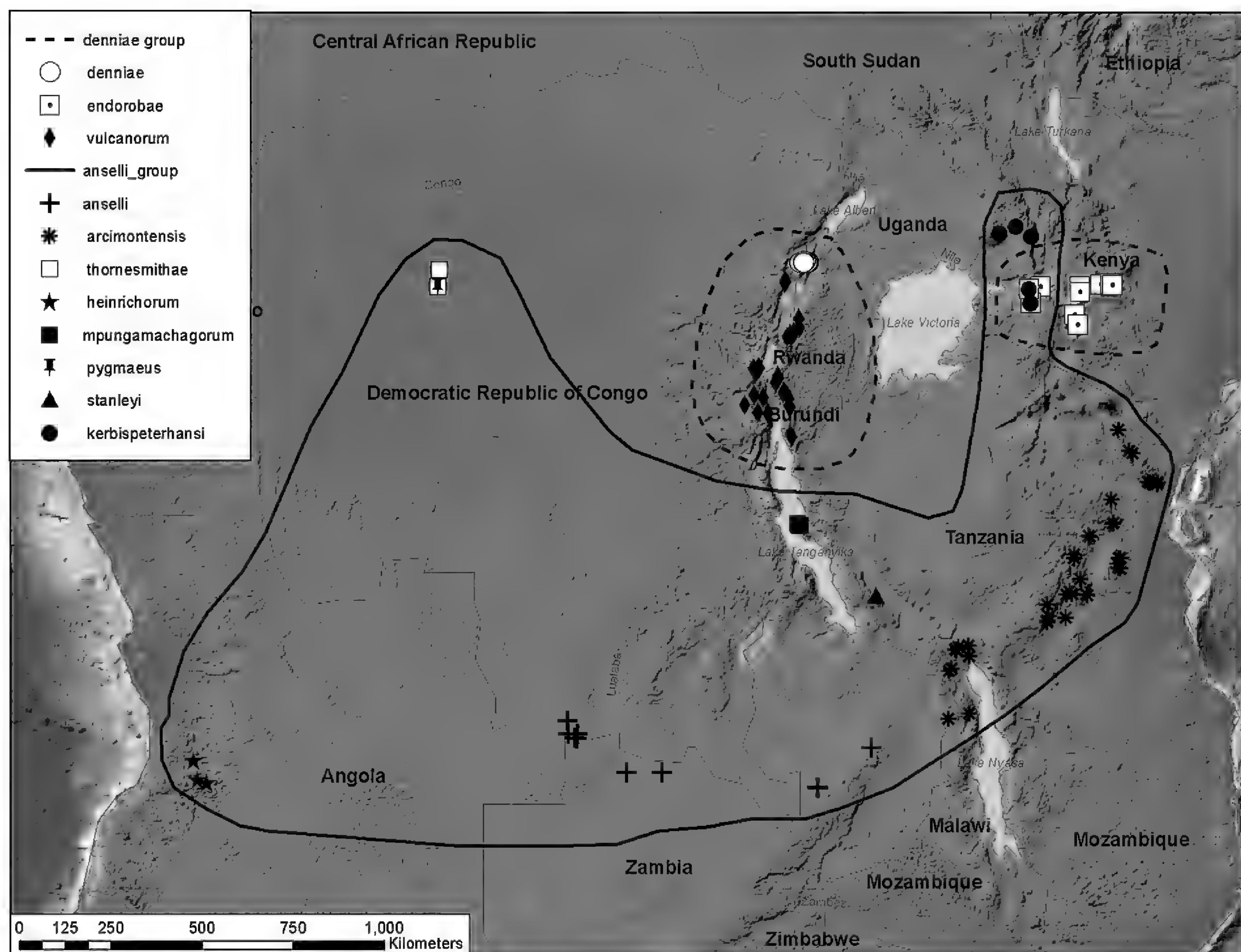
*urn:lsid:zoobank.org:act:1B7A9BDE-24A0-47DE-9B66-F3B40D95EE54*

*Dendromus* sp. – Doty et al. (2017)

**Holotype.** Field Museum of Natural History, Division of Mammals number FMNH 219684 (field number WT Stanley 11,575; CDC 207), collected by W.T. Stanley, 13 June 2012 (listed in field notes as *Dendromus* sp.) during the first small mammal survey in the area. The specimen, consisting of a study skin and skull with carcass in alcohol, is a young adult female with first upper molar in early wear (stage IV of Verheyen & Bracke 1966). The basisphenoid-occipital suture is unfused. External mea-

**Table 3.** Uncorrected cytochrome-*b* *p*-distances (%) within (bolded numbers on diagonal) and between *Hylomyscus anselli* group species, calculated in MEGA 7.0.26 (Kumar et al. 2016).

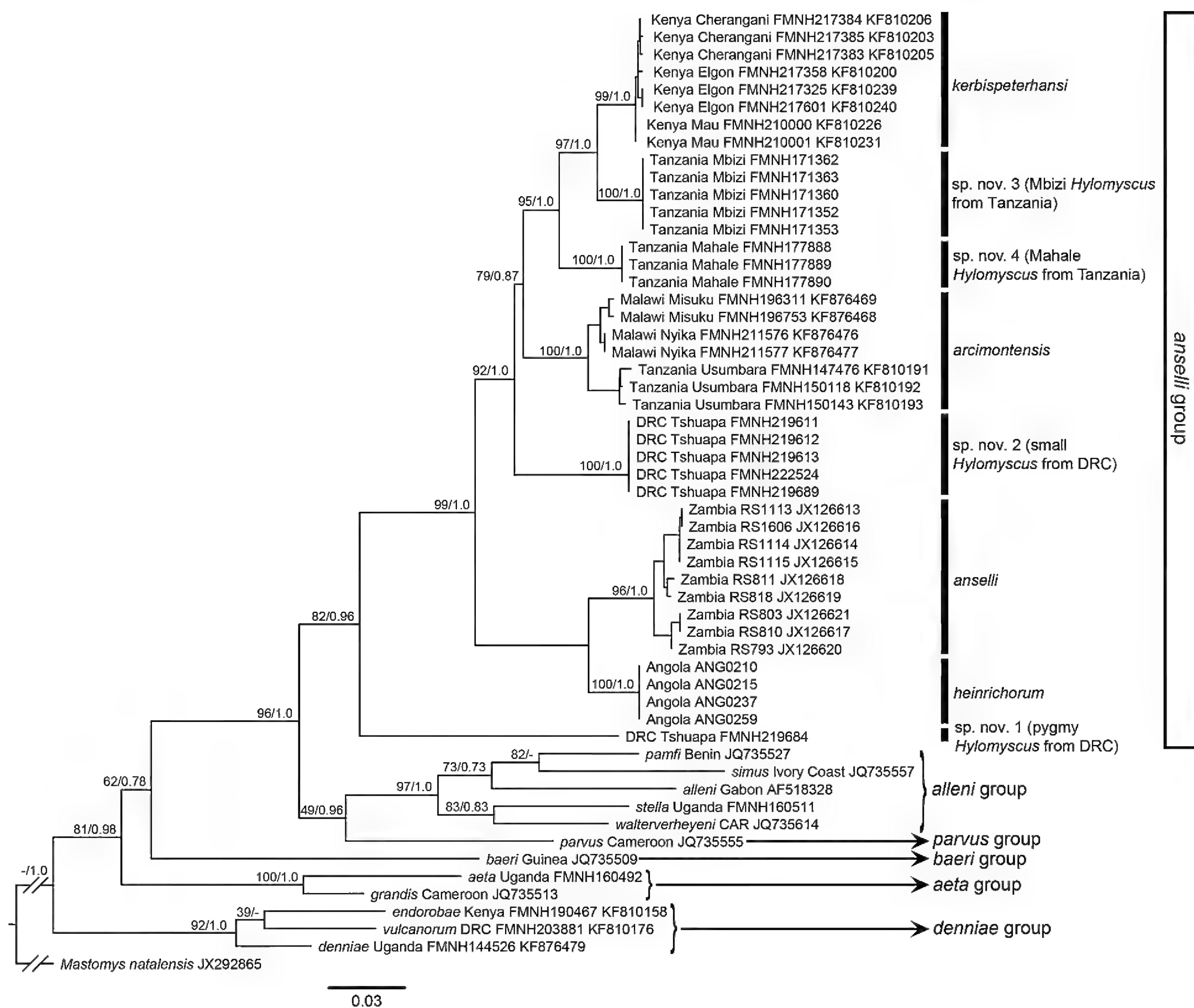
Taxon	1	2	3	4	5	6	7	8
1 <i>anselli</i>	<b>1.1</b>							
2 <i>arcimontensis</i>	8.1	<b>1.5</b>						
3 <i>mpungamachagorum</i> sp. nov.	8.2	6.0	<b>0.0</b>					
4 <i>heinrichorum</i>	4.4	7.9	8.4	<b>0.3</b>				
5 <i>kerbispeterhansi</i>	8.1	6.6	4.7	8.0	<b>0.0</b>			
6 <i>stanleyi</i> sp. nov.	8.2	6.3	4.9	9.3	3.2	<b>0.0</b>		
7 <i>thornesmithae</i> sp. nov.	8.6	6.6	6.6	8.3	6.9	6.4	<b>0.0</b>	
8 <i>pygmaeus</i> sp. nov.	11.1	10.9	10.9	10.9	10.8	11.5	12.1	<b>na</b>



**Fig. 3.** Map of the distributions of members of the *Hylomyscus denniae* and *Hylomyscus anselli* groups.

surements were made in the field: TL 132, TV 76, HF 14, EL 12, Wt 5.8. Specimen caught in a pitfall trap (PF 4, Bucket 10).

**Type locality.** Democratic Republic of Congo, Tshuapa Province, 4 km N of Boende, Baliko (0.24127 S, 20.8833 E), right side Tshuapa River, elevation of 358 m.



**Fig 4.** Phylogenetic tree, using cytochrome-*b*, of the genus *Hylomyscus* with focus on the *Hylomyscus anselli* group. Bootstrap values for maximum likelihood analysis followed by posterior probabilities for Bayesian analysis are indicated above branches.

**Diagnosis.** Easily recognized by its small size: HB 56, Wt 5.8, CI 15.9, CLM 2.6, WM1 0.8. All are significantly smaller than any other members of the *H. anselli* group (Figs 5a, c, 6a, c, e; Tables 4–5), or, for that matter, any member of *Hylomyscus*. Ears exceptionally long (Fig. 5c), 23% of HB. Incisors slightly pro-odont (Fig. 6e). Braincase inflated, round and bulbous (Figs 6a, e). Rostrum extremely short (Fig. 6a, LN/ONL = 26.7%).

**Comparisons.** This species is by far the smallest member of the *Hylomyscus anselli* group as reflected in Tables 4–5: e.g., crown length of upper molars 2.6, compared to 3.0–4.5; HB 56, compared to 76–109 for other members of the group.

**Description.** Size very small (HB 56, mass 5.8). Tail 36% longer than HB. Tail unicolor with 21–23 annulations per cm. Ears long, 23% of HB, 13 mm re-measured from dry study skin (vis a vis field notes = 12 mm). Belly hairs 3 mm, basal 50% slate grey, distal 50% slightly ochraceous. Dorsal hairs 4 mm, basal 2.5 mm slate grey, tipped with carmel brown. Dorsum of head appearing more grey, due to shorter ochraceous tips (perhaps in molt). Upper lip creamy white. Vibrissae ‘long’ – up to 25 mm in length –, ventral vibrissae white; dorsal vibrissae black and shorter. Young adult female with mammae not visible on dry skin. The number of fleshy pads on the hind foot are not determinable from the single study skin. The number of fleshy palatal ridges are not visible in the single cleaned skull.

**Table 4.** Craniodental measurements in millimeters (mean  $\pm$  1 SD and range) for eight species in the *Hylomyscus anselli* group. Abbreviated variables are defined in the text.

Variable	<i>kerbispeterhansi</i> (n = 51)	<i>stanleyi</i> (n = 22)	<i>mpungamachagorum</i> (n = 4)	<i>arcimontensis</i> (n = 53)	<i>thornesmithae</i> (n = 5)	<i>anselli</i> (n = 4)	<i>heinrichorum</i> (n = 13)	<i>pygmaeus</i> (n = 1)
ONL	26.36 $\pm$ 0.75 (24.68–27.96)	27.16 $\pm$ 0.56 (26.20–28.48)	25.25 $\pm$ 0.08 (25.17–25.36)	25.69 $\pm$ 0.69 (24.12–27.25)	22.84 $\pm$ 0.61 (22.09–23.39)	25.98 $\pm$ 0.30 (25.74–26.39)	26.73 $\pm$ 0.79 (25.52–28.12)	17.45
CI	24.73 $\pm$ 0.87 (22.66–26.53)	24.84 $\pm$ 0.56 (23.60–26.42)	23.34 $\pm$ 0.31 (22.95–23.70)	23.80 $\pm$ 0.70 (22.43–25.61)	21.42 $\pm$ 0.47 (21.06–22.08)	23.98 $\pm$ 0.57 (23.28–24.68)	24.77 $\pm$ 0.75 (23.68–26.18)	15.89
ZB	13.19 $\pm$ 0.42 (12.25–14.19)	13.35 $\pm$ 0.25 (12.86–13.95)	12.65 $\pm$ 0.12 (12.48–12.73)	12.85 $\pm$ 0.45 (11.91–13.89)	11.66 $\pm$ 0.10 (11.55–11.80)	12.84 $\pm$ 0.22 (12.53–13.05)	12.99 $\pm$ 0.38 (12.52–13.70)	9.68
BBC	11.81 $\pm$ 0.27 (11.25–12.51)	12.14 $\pm$ 0.24 (11.62–12.48)	11.65 $\pm$ 0.16 (11.51–11.83)	11.66 $\pm$ 0.34 (10.90–12.47)	11.07 $\pm$ 0.49 (10.64–11.62)	11.80 $\pm$ 0.39 (11.24–12.12)	11.81 $\pm$ 0.22 (11.55–12.29)	9.27
BOC	6.10 $\pm$ 0.18 (5.58–6.45)	6.11 $\pm$ 0.12 (5.93–6.32)	5.92 $\pm$ 0.11 (5.78–6.05)	6.12 $\pm$ 0.22 (5.55–6.54)	5.62 $\pm$ 0.17 (5.42–5.81)	6.18 $\pm$ 0.18 (6.00–6.40)	6.26 $\pm$ 0.12 (6.06–6.43)	4.94
IO	4.35 $\pm$ 0.10 (4.17–4.57)	4.60 $\pm$ 0.12 (4.46–4.91)	4.52 $\pm$ 0.21 (4.34–4.80)	4.36 $\pm$ 0.15 (4.09–4.66)	3.89 $\pm$ 0.08 (3.77–3.99)	4.41 $\pm$ 0.16 (4.20–4.57)	4.53 $\pm$ 0.08 (4.42–4.69)	3.74
LN	9.45 $\pm$ 0.46 (8.44–10.69)	10.14 $\pm$ 0.33 (9.26–10.61)	8.71 $\pm$ 0.07 (8.64–8.81)	9.19 $\pm$ 0.35 (8.44–9.76)	7.69 $\pm$ 0.31 (7.32–7.97)	9.13 $\pm$ 0.27 (8.81–9.38)	9.16 $\pm$ 0.49 (8.06–9.94)	4.66
BR	4.56 $\pm$ 0.27 (4.16–5.60)	4.55 $\pm$ 0.14 (4.27–4.97)	4.43 $\pm$ 0.11 (4.27–4.50)	4.45 $\pm$ 0.20 (4.08–4.96)	4.11 $\pm$ 0.20 (3.94–4.42)	4.59 $\pm$ 0.07 (4.48–4.63)	4.35 $\pm$ 0.24 (4.01–4.73)	3.23
PPL	9.03 $\pm$ 0.44 (8.12–10.32)	9.36 $\pm$ 0.27 (8.75–10.05)	8.98 $\pm$ 0.24 (8.69–9.28)	8.96 $\pm$ 0.31 (8.37–9.63)	8.62 $\pm$ 0.33 (8.23–8.94)	9.11 $\pm$ 0.24 (8.87–9.45)	9.40 $\pm$ 0.44 (8.58–10.20)	6.28
LBP	4.50 $\pm$ 0.18 (3.92–4.81)	4.76 $\pm$ 0.18 (4.36–5.09)	4.26 $\pm$ 0.06 (4.19–4.34)	4.42 $\pm$ 0.21 (3.95–4.83)	4.18 $\pm$ 0.14 (4.00–4.37)	4.79 $\pm$ 0.17 (4.60–4.93)	4.45 $\pm$ 0.20 (4.08–4.77)	2.91
LIF	5.59 $\pm$ 0.25 (5.02–6.06)	5.48 $\pm$ 0.16 (5.29–5.78)	5.09 $\pm$ 0.19 (4.89–5.34)	5.38 $\pm$ 0.30 (4.78–6.11)	4.38 $\pm$ 0.22 (4.10–4.67)	5.23 $\pm$ 0.22 (5.09–5.55)	5.84 $\pm$ 0.27 (5.33–6.33)	2.83
LD	7.82 $\pm$ 0.32 (7.25–8.62)	7.59 $\pm$ 0.17 (7.38–8.03)	6.95 $\pm$ 0.19 (6.74–7.20)	7.22 $\pm$ 0.25 (6.76–7.83)	6.55 $\pm$ 0.29 (6.14–6.82)	7.19 $\pm$ 0.19 (7.03–7.41)	7.47 $\pm$ 0.35 (7.05–8.15)	4.39
BZP	2.39 $\pm$ 0.12 (2.16–2.75)	2.28 $\pm$ 0.13 (2.07–2.57)	2.30 $\pm$ 0.05 (2.24–2.36)	2.31 $\pm$ 0.12 (2.01–2.54)	1.90 $\pm$ 0.09 (1.79–2.00)	2.34 $\pm$ 0.14 (2.17–2.47)	2.64 $\pm$ 0.17 (2.31–2.80)	1.76
LAB	4.59 $\pm$ 0.21 (4.26–5.23)	4.51 $\pm$ 0.12 (4.28–4.74)	4.23 $\pm$ 0.13 (4.04–4.34)	4.23 $\pm$ 0.12 (3.98–4.43)	3.86 $\pm$ 0.14 (3.73–4.05)	4.55 $\pm$ 0.07 (4.48–4.63)	4.45 $\pm$ 0.11 (4.23–4.61)	3.23
CLM	3.80 $\pm$ 0.07 (3.66–3.93)	4.13 $\pm$ 0.12 (3.97–4.51)	3.78 $\pm$ 0.05 (3.74–3.85)	3.85 $\pm$ 0.18 (3.40–4.17)	3.16 $\pm$ 0.09 (3.02–3.25)	4.10 $\pm$ 0.09 (3.97–4.18)	4.17 $\pm$ 0.14 (3.99–4.52)	2.60
WM1	1.20 $\pm$ 0.04 (1.12–1.29)	1.24 $\pm$ 0.04 (1.14–1.30)	1.13 $\pm$ 0.01 (1.12–1.13)	1.18 $\pm$ 0.05 (1.06–1.28)	0.95 $\pm$ 0.09 (0.86–1.06)	1.24 $\pm$ 0.04 (1.19–1.28)	1.28 $\pm$ 0.06 (1.17–1.34)	0.82

Skull tiny (ONL 17.45, CRM 2.6). Rostrum exceptionally short (LN/ONL = 26.7%) but this is expected to increase in older individuals. Inter-orbital region proportionately broad. Upper incisors slightly pro-odont. Incisive foramina fall short of upper tooth row. T3 on  $M^2$  is present but a tiny vestige. Braincase bulbous and dorso-ventrally inflated. Hamular process of the squamosal long and thin, providing for a very large subsquamosal fenestra which is about 40% the size of the postglenoid foramen (see Carleton & Stanley 2005: fig. 6). Maxillo-palatal suture located at the rear third of the  $M^1$  (Figs 6c, 8a). Post palatal foramina large, starting between  $M^1$  and  $M^2$  and extend back to middle of  $M^2$  (Figs 6c, 8a). Fronto-parietal suture broadly U-shaped. Zygomatic plate narrow (1.34 mm) and without any sinuosity but gently sloping

forward throughout. Mesopterygoid fossa rounded and open widely at rostral end.

As a divergent member of the *Hylomyscus anselli* group, some of the following characters contrast the Carleton et al. list of characters (2006: table 7) defining this group: 1) pectoral mammae unknown (not available as the holotype is a young adult), 2) upper incisors slightly pro-odont (Fig. 6e), whereas Carleton et al. (2006) characterized the *H. anselli* group as opisthodont, 3) T3 on  $M^1$  is distinct and sub equal with t1 rendering it ‘large’ per Carleton et al. (2006); the anterior chevron is more or less symmetrical (Fig. 7g), whereas Carleton et al. (2006) characterized the ‘*H. anselli*’ group as having a ‘medium’ sized t3 (e.g., smaller than t1), 4) t9 on  $M^1$  is distinct (Fig. 7g), whereas Carleton et al. (2006) characterized the

**Table 5.** External measurements (mean  $\pm$  SD, range, and sample size) of all eight members of the *Hylomyscus anselli* group. Measurements are in millimeters and mass is in grams. Abbreviated variables are defined in the text. \*See text, totals have 1.0 mm added.

Variable	<i>kerbis peterhansi</i>	<i>stanleyi</i>	<i>mpungamachagorum</i>	<i>arcimontensis</i>	<i>thornesmithae</i>	<i>anselli</i>	<i>heinrichorum</i>	<i>pygmaeus</i>
TOT	230.2 $\pm$ 12.1 (201–260) 50	245.2 $\pm$ 7.1 (233–264) 32	234.3 $\pm$ 6.6 (230–244) 4	227.3 $\pm$ 12.5 (205–262) 50	204.4 $\pm$ 7.6 (193–212) 5	222.4 $\pm$ 13.4 (205–254) 16	233.3 $\pm$ 12.6 (207–255) 21	132
HB	92.6 $\pm$ 5.6 (80–103) 50	101.7 $\pm$ 3.8 (93–109) 32	97.8 $\pm$ 1.9 (95–99) 4	91.3 $\pm$ 5.6 (77–104) 50	83.2 $\pm$ 4.4 (76–87) 5	87.2 $\pm$ 6.4 (78–101) 16	94.8 $\pm$ 6.8 (82–106) 21	56
TAIL	137.6 $\pm$ 8.8 (117–158) 50	145.5 $\pm$ 5.1 (136–159) 32	138.3 $\pm$ 6.3 (132–147) 4	136.0 $\pm$ 9.0 (115–161) 50	121.2 $\pm$ 3.3 (117–125) 5	135.2 $\pm$ 9.2 (121–153) 16	138.5 $\pm$ 7.4 (121–150) 21	76
HF	20.4 $\pm$ 0.9 (19–22) 49	21.1 $\pm$ 0.8 (20–23) 32	20.3 $\pm$ 0.5 (20–21) 4	20.5 $\pm$ 1.0 (18–22) 50	18.0 $\pm$ 1.0 (17–19) 5	20.7* $\pm$ 0.5 (19.5–21.5)* 24	22.0* $\pm$ 0.9 (19.5–23)* 21	14
EAR	19.8 $\pm$ 1.0 (17.5–22) 47	19.9 $\pm$ 0.8 (18–21) 32	19.0 $\pm$ 0.0 (19) 4	18.2 $\pm$ 1.0 (17–21) 50	14.8 $\pm$ 0.8 (14–16) 5	18.0 $\pm$ 0.7 (16.5–19) 24	18.6 $\pm$ 1.0 (17–21) 21	13
Wt	24.9 $\pm$ 4.0 (17–39) 48	27.6 $\pm$ 3.1 (22–34.5) 31	21.8 $\pm$ 1.2 (20.5–23) 4	22.0 $\pm$ 3.7 (15–29.5) 50	13.9 $\pm$ 1.4 (11.5–15) 5	20.4 $\pm$ 4.0 (16–30.5) 15	NA	5.8
TAIL/HB	1.5 $\pm$ 0.1 (1.2–1.7) 50	1.4 $\pm$ 0.0 (1.4–1.5) 32	1.4 $\pm$ 0.1 (1.3–1.5) 4	1.5 $\pm$ 0.1 (1.2–1.7) 50	1.4 $\pm$ 0.1 (1.4–1.5) 5	1.6 $\pm$ 0.1 (1.4–1.8) 16	1.5 $\pm$ 0.1 (1.3–1.7) 21	1.4

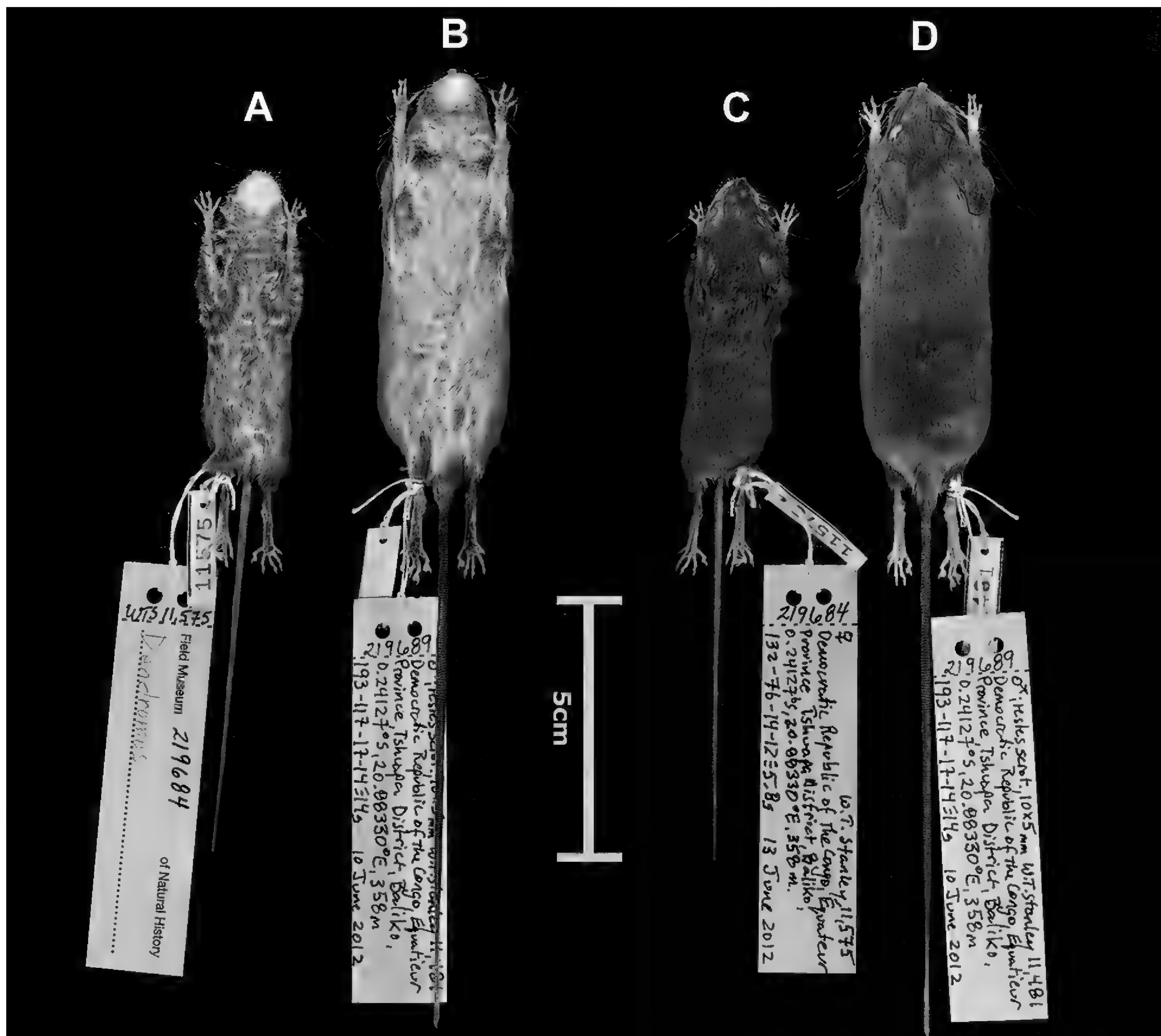
‘*H. anselli*’ group as ‘indistinct’ 5) interorbital constriction is amphoral, whereas Carleton et al. (2006) characterized the ‘*H. anselli*’ group as having a ‘weak shelf, 6) rostral length is extremely short (Fig. 6a), whereas Carleton et al. (2006) characterized the ‘*H. anselli*’ group with a ‘medium’ length rostrum, 7) incisive foramen is short, falling well short of the alveoli of  $M^1$  (Figs 6c, 8a) as opposed to the Carleton et al. (2006) characterization as ‘medium’ (reaching anterior root of  $M^1$ ), 8) the hamular strap is long and thin, subsquamosal foramen is large in size (see Carleton & Stanley 2005: fig. 6; Fig. 6e). In sum, several characters of this new species are unique or align more with the *Hylomyscus alleni* group than the *H. anselli* group: more proodont, distinct T9 on  $M^1$ , amphoral inter-orbital region, extreme shortening of the rostrum, and shorter incisive foramina. Perhaps these contrasts are not surprising given the basal position of this taxon.

**Ecology.** The habitat is seasonally flooded primary forest, ‘edaphic forest’ (Verhegghen et al. 2012). However, the pitfall line was set in a drier part of the forest and was less subject to flooding. The area supports two dry seasons (January to early March, and June to early September) with the rest being rainy averaging ca. 210 cm per year. Daily temperature average between 24 °C and 30 °C (Doty et al. 2017). Type specimen was caught in a generally dry area of the forest.

The vegetation of the Tshuapa region is mainly characterized by semperfervent or semi-sempervent rain forests bound to hydromorphic soils, secondary forests

and grassy vegetation (Evrard 1968). The semperfervent rain forests of terra-firma are distinguished by their structural density, distinct stratification and epiphytism (Lebrun & Gilbert 1954), while the upper stratum can reach 40–45 m in height. There are two types of forest bound to hydromorphic soils in swampy zones. These include periodically flooded forests (including where the type specimen was collected) comprising the following species: *Parinari congoensis*, *Guibourtia demeusei*, *Zeyrrhella longipedisellata* and swampy forests composed of *Entandrophragma palustre*, *Alstonia congoensis*, *Coelocaryon botryodes*, and *Sterculia tragacantha*. The second forest type is composed of bushy forests along the banks of large rivers including *Alchornea cardifolia*, *Lacosperma secundiflorum*; waterside forests with *Coelocaryon botryodes*, *Erispermum microspermum*, *Sclerosperma manirii*, and *Cleistanthus mycrophyllus*. Secondary forests are found around villages, roads and on former sites of forest extraction. Species frequently observed are *Musanga cecropioides*, *Harungana madagascariensis*, *Trema orientalis*, *Oncoba subtomentosa*, *Pycnanthus angolensis*, *Petersianthus macrocarpus*, *Ricinodendron heudelotii*, *Canarium schweinfurthii*, *Alstonia boonei*, and *Elaeis guineensis*. Grassy vegetation results from forest degradation and includes frequently burnt fallow fields, mainly with Graminaceae of the genera *Panicum*, *Pennisetum*, *Imperata*, *Serata*, and *Sorghum* (Evrard 1968).

**Reproduction.** The sole specimen is a young female with teats that are neither developed nor visible.



**Fig. 5.** Skins of two new species of the *Hylomyscus anselli* group from the Democratic Republic of Congo. (a) ventral and (c) dorsal views of *Hylomyscus pygmaeus* sp.nov., and (b) ventral and (d) dorsal views of *Hylomyscus thornesmithae* sp.nov.

**Etymology.** Named for its diminutive size. We recommend “pygmy wood mouse” as an English common name.

***Hylomyscus thornesmithae* sp. nov.** Kerbis Peterhans, Hutterer & Demos

urn:lsid:zoobank.org:act:DAE95BEF-D424-42C2-8415-A1805F494ADC  
*Hylomyscus* sp. – Doty et al. (2017)

**Holotype.** Field Museum of Natural History, Division of Mammals number FMNH 222524 (field number WT Stanley 11,664; CDC 746), collected by W.T. Stanley, 27 June 2013 (originally listed as *Hylomyscus* sp.). The type specimen, consisting of an alcoholic carcass with skull removed, is an adult female with first upper molar in ear-

ly wear (advanced age stage IV) and large teats (2+4). The basisphenoid-occipital suture is fused. External measurements were made in the field: TL 210, TV 124, HF 18, EL 15, Wt 15. Specimen caught in a standard snap trap; apparently held by tail in trap as tail is broken  $\frac{1}{3}$  way down. Type specimen captured just behind camp in secondary growth forest on the edge of a forest that may occasionally be subject to flooding.

**Type locality.** Democratic Republic of Congo, Tshuapa Province, rt side Tshuapa River, 14 km north of Boende by road, Quatorz ( $0.16919^{\circ}$  S,  $20.92611^{\circ}$  E) at an elevation of 322 m.



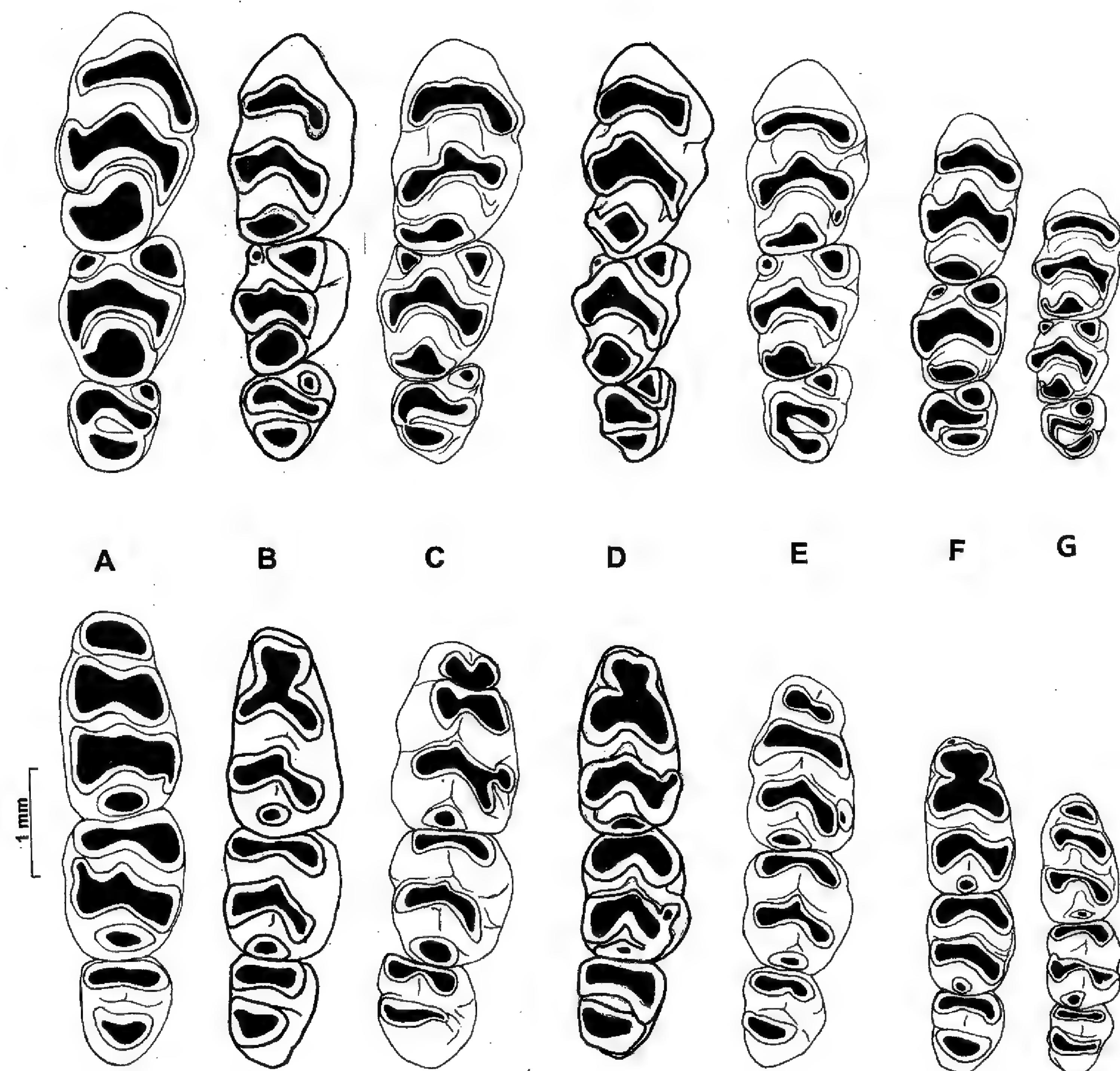
**Fig. 6.** Skulls of two new species of the *Hylomyscus anselli* group from the Democratic Republic of Congo. (a) Dorsal (c) ventral and (e) lateral views of *Hylomyscus pygmaeus* sp.nov. (b) Dorsal, (d) ventral and (f) lateral views of *Hylomyscus thornesmithae* sp. nov.

**Paratypes** ( $n = 4$ ). All caught in conventional trap lines from Democratic Republic of Congo, Tshuapa Province, 4 km N of Boende, Baleko ( $0.24127^\circ$  S,  $20.8833^\circ$  E) at an elevation of 358 m: FMNH 219611 (WTS 11471, CDC 103), alcoholic carcass with extracted skull, old scrotal male; FMNH 219612 (WTS 11592, CDC 224) alcoholic carcass with extracted skull, old female, teats 2+4; FMNH 2119613 (WTS 11594, CDC 226), alcoholic carcass with extracted skull, old scrotal male; FMNH 219689 (WTS 11481, CDC 113), skin and skull with carcass preserved in alcohol, old scrotal male, testes 10x5.

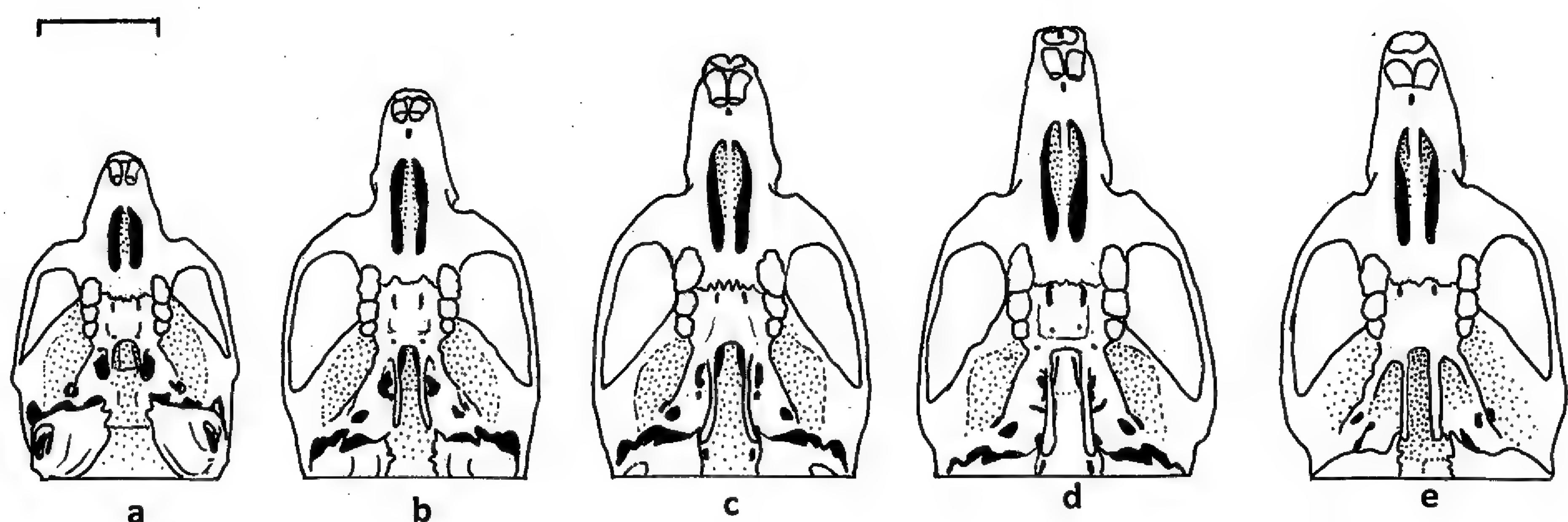
**Diagnosis.** Easily differentiated within the *Hylomyscus anselli* group by its small size (second smallest, but still much larger than preceding species): HB 83 (mean), Wt 13.9 (mean), ONL 22.8 (mean), CLM 3.2 (mean). All are significantly smaller than all other members of the *H. anselli* group (Tables 4–5), excepting the previously described species.

**Comparisons.** Upper incisors orthodont, contrasting with the proodont condition in *Hylomyscus pygmaeus*. Crown length of upper molars 3.0–3.25 mm, much larger than *Hylomyscus pygmaeus* (under 2.6 mm). External and cranio-dental measurements smaller than all other members of the *H. anselli* group (excepting *Hylomyscus pygmaeus*).

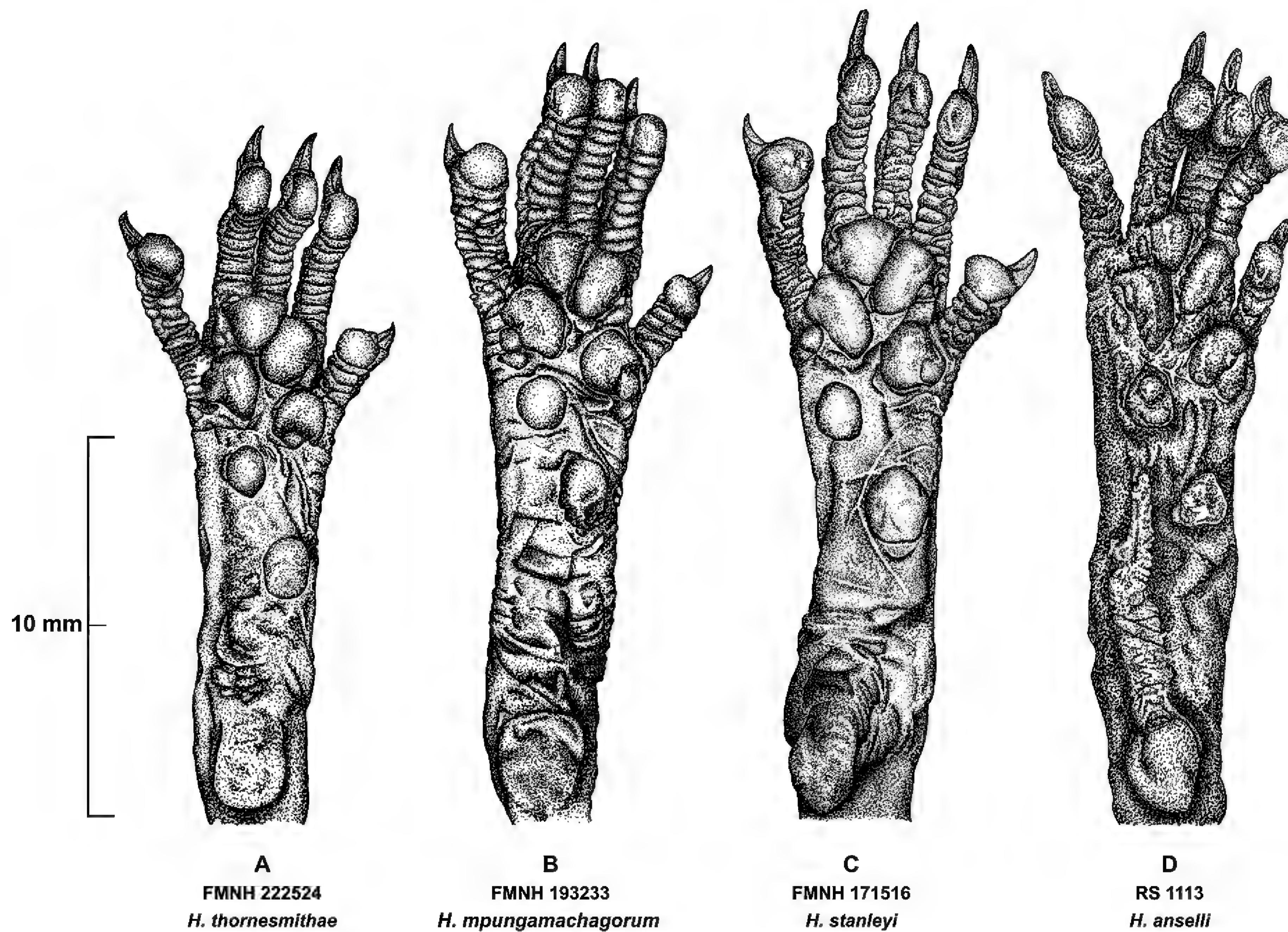
**Description.** Size very small (mean HB = 83, mean mass = 13.9). Tail 46% longer than HB, unicolor with ca. 18 annulations per cm. Ears of normal size, 18% of HB; ear color dark grey. Belly hairs 5 mm, basal 3 mm slate grey, distal 2 mm white. Dorsal hairs 7 mm, basal 5 mm slate grey, apical 2 mm orange, more bright orange towards flanks. Vibrissae up to 33 mm in length, mostly black but with 2–3 white hairs; upper lip with white fur patch behind vibrissae. Teats 2+4. The hind foot possesses the standard murine complement of 6 pads (see Ibe et al. 2014: fig. 2, II for reference); there is a single accessory pad on the 1<sup>st</sup> and 4<sup>th</sup> interdigital pads; the first is clear and well-defined while the 4<sup>th</sup> is larger and more integrat-



**Fig. 7.** Sketches of right upper (top row) and lower tooth rows (bottom row) of members of the *Hylomyscus anselli* group, including four new species described herein: (a) *H. stanleyi* sp. nov. (FMNH 171512), (b) *H. anselli* (BMNH 74.250), (c) *H. kerbispeterhansi* (FMNH 209995), (d) *H. mpungamachagorum* sp. nov. (FMNH 177889), (e) *H. arcimontensis* (FMNH 147271), (f) *H. thornesmithae* sp. nov. (FMNH 222524), (g) *H. pygmaeus* sp. nov. (FMNH 219684). Scale = 1 mm.



**Fig. 8.** Sketches of the bony palates of *Hylomyscus anselli* and the four new species of the *Hylomyscus anselli* group: (a) *H. pygmaeus* sp. nov. FMNH 219684, (b) *H. thornesmithae* sp. nov. FMNH 222524, (c) *H. mpungamachagorum* sp. nov. FMNH 177889, (d) *H. stanleyi* sp. nov. FMNH 171362, and (e) *H. anselli* BMNH 74.250. Scale = 5 mm.



**Fig. 9.** Sketches of feet and plantar tubercles of four species of the *Hylomyscus anselli* group: (a) *H. thornesmithae* sp. nov., (b) *H. mpungamachagorum* sp. nov., (c) *H. stanleyi* sp. nov., and (d) *H. anselli*.

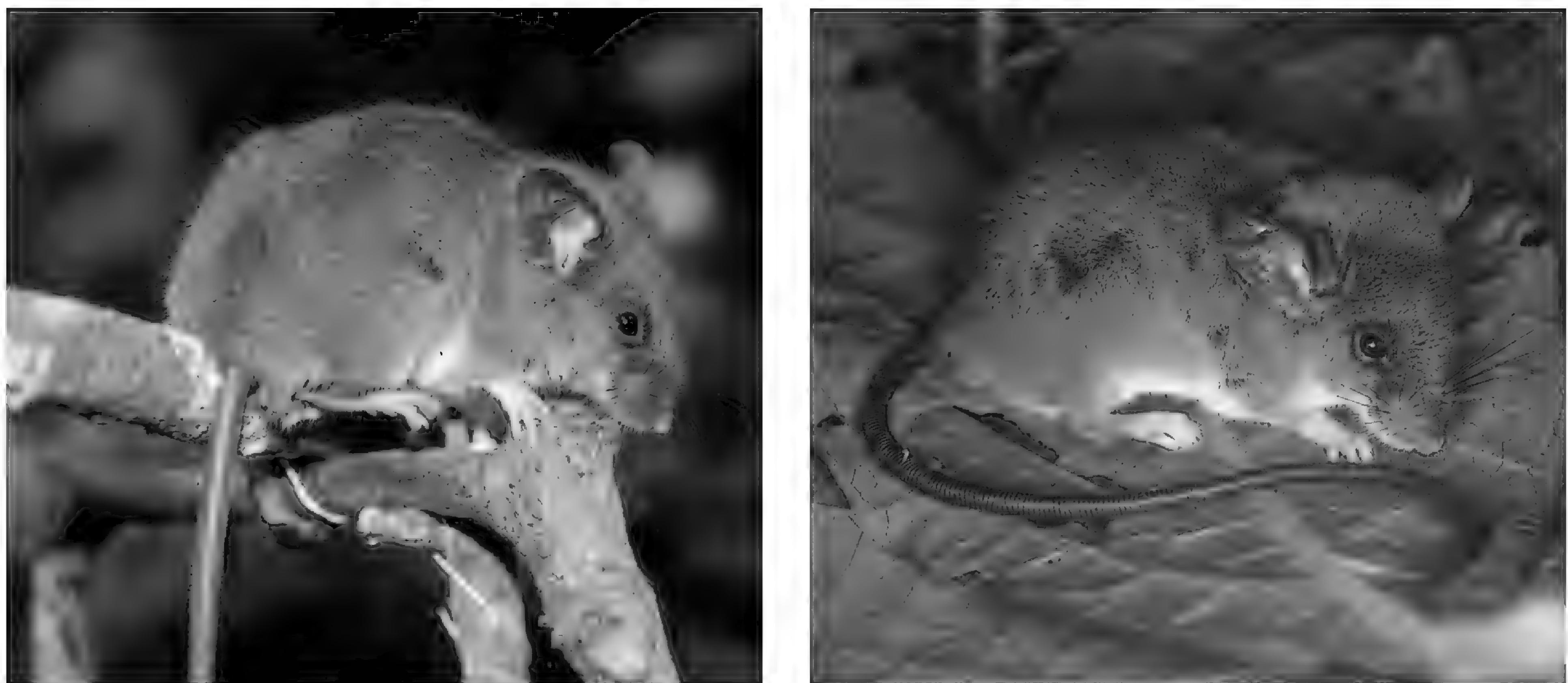
ed (Fig. 9a). The number of fleshy palatal ridges are not visible on any of the prepared skulls.

Skull small (mean ONL = 22.8, mean CRM = 3.2). Rostrum short, LN/ONL = 33.7%. Upper incisors orthodont. Incisive foramina fall well short of upper tooth row. T3 on M<sup>2</sup> is tiny. Braincase elongated. Hamular process of the squamosal long and thin, providing for a large subsquamosal fenestra which is about 35% the size of the post glenoid foramen (see Carleton & Stanley 2005: fig 6; Fig 6f). Maxillo-palatal suture zig-zags through the middle of the M<sup>1</sup> (Figs 6d, 8b). Post palatal foramina large, starting at rear 3<sup>rd</sup> of M<sup>1</sup> or between M<sup>1</sup> and M<sup>2</sup> and continues through to the 1<sup>st</sup> third of M<sup>2</sup>. Zygomatic plate slightly sinuous. In aged individuals (FMNH 219611, FMNH 219612), the incisive foramina fall even shorter of the UTR, the maxillo-palatal suture is located more forward at the first half of M<sup>1</sup> and the post palatal foramina are more forward at the rear half of the M<sup>1</sup>. Frontoparietal suture broadly rounded, U-shaped. Zygomatic plate narrow (1.9 mm) and virtually orthogonal to skull profile but gently sloping forward in lower third. Mesopterygoid fossa rounded at rostral end.

As a member of the *Hylomyscus anselli* group (sensu Carleton et al. 2006: table 7), the following characters are relevant: 1) mammae: 2+4, 2) upper incisors orthodont (Fig. 6f), 3) T3 on M<sup>1</sup> is ‘medium’ in size (smaller than t1), the anterior chevron is moderately asymmetrical (Fig. 7f), 4) T9 on M<sup>1</sup> is distinct but reduced (Fig. 7f), 5) interorbital constriction is amphoral in shape (Fig. 6b), 6) rostral length is short, LN/ONL = 33.7% (Fig. 6b), 7) incisive foramen is very short, falling short of the roots of M1 (Figs 6d, 8b), 8) the hamular strap is short but thin and delicate and allows for a large subsquamosal foramen, which is about 25% the size of the postglenoid foramen (Fig. 6f).

**Distribution.** Known only from two locations, both are ca. 250 km S of the Congo River, off the right bank of the Tshuapa River, Tshuapa Province, Democratic Republic of Congo. In addition to the type locality (n = 1), four paratypes are from 4 km N of Boende at Baleko (0.24127° S, 20.8833° E, elevation 358 m).

**Ecology.** Type specimen and 3 paratypes all caught in standard mammal snap traps while the fifth was caught



**Fig. 10.** Live photos of two new species of the *Hylomyscus anselli* group from Tanzania: (left) *H. stanleyi* sp. nov. and (right) *H. mpungamachagorum* sp. nov.

in a Sherman live trap. Type specimen captured in regenerating secondary forest on the edge of a seasonally flooded forest. One specimen captured in a trap line that was never flooded during heavy rain events while three came from a trap line that was prone to flooding.

**Reproduction.** All animals captured ( $n = 5$ ) were adult. Type is adult female with swollen teats 2 + 4. A second female (FMNH 219612), despite having well-worn molars (beyond stage VII), had small teats that are difficult to decipher. Three adult males all with scrotal testes; testes of FMNH 219689 measured 10 × 5 in the field.

**Etymology.** Ellen Thorne Smith was a “professional volunteer” serving 2–3 days per week, sorting and organizing the bird collections at the Field Museum, and conducting original published research from the mid 1930’s until the 1970’s. During World War II with the museum’s ornithologists away in Washington, she ran the Division of Ornithology. We recommend “Mother Ellen’s wood mouse” as an English common name.

***Hylomyscus stanleyi* sp. nov.** Kerbis Peterhans, Hutterer & Demos

*urn:lsid:zoobank.org:act:4512E5DE-2139-46CB-B2F9-823C9C3B1BAD*  
*Hylomyscus anselli* – Carleton & Stanley (2005); Carleton, Kerbis Peterhans & Stanley (2006); Demos, Agwanda & Hickerson (2014a); Carleton, Banasiak & Stanley (2015)

*Hylomyscus cf. anselli* – Nicolas et al. (2020)

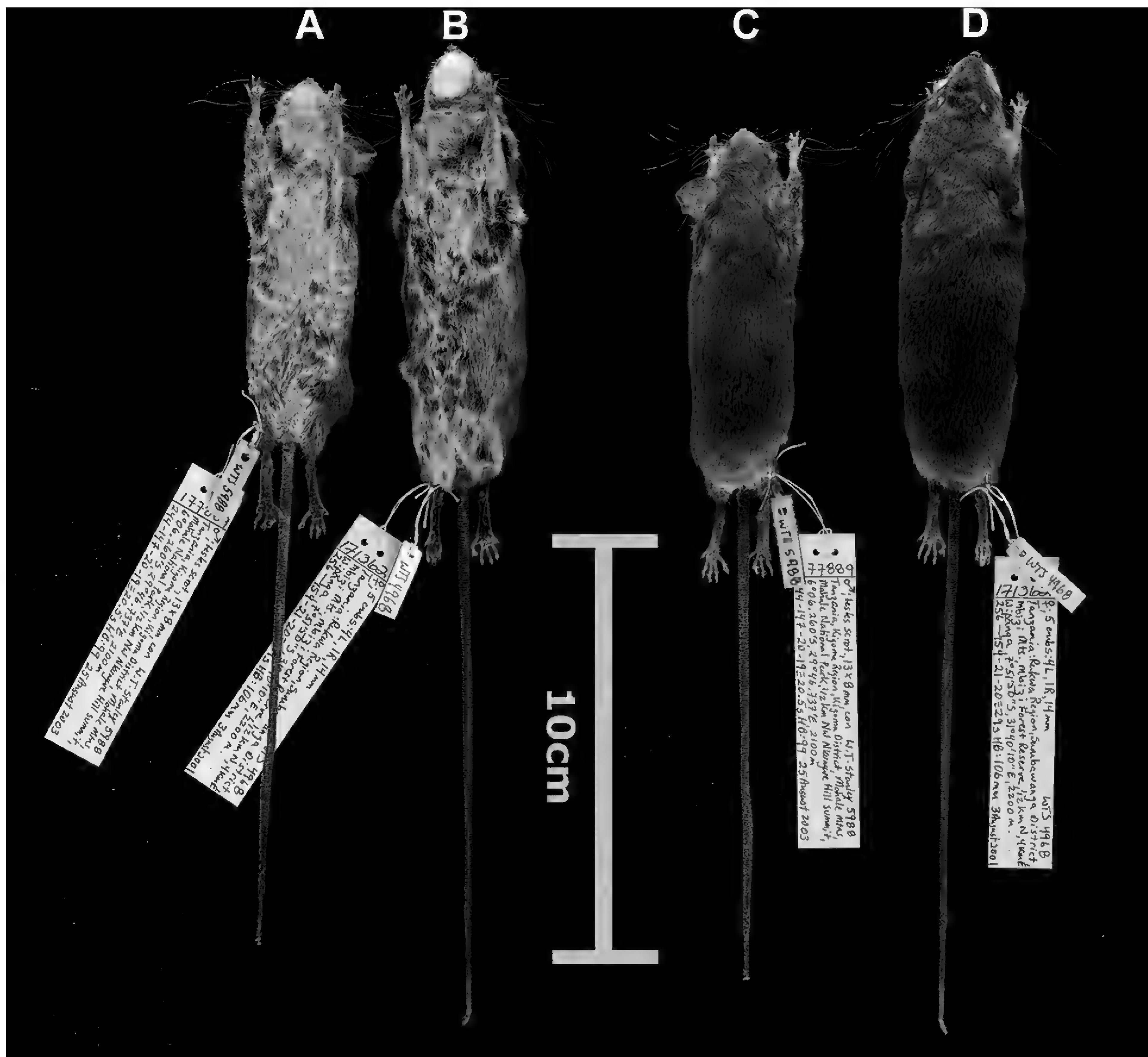
**Holotype.** Field Museum of Natural History, Division of Mammals number FMNH 171362 (field number WT Stanley 4968), collected by Cosmos, 03 August 2001

(originally listed as *Hylomyscus* sp.). The specimen, consisting of a skin, skull and alcoholic carcass, is an adult pregnant female with first upper molar well worn (stage IV of Verheyen & Bracke 1966). Teats 2+4, large, embryos 4L, 1R (CR 14). The holotype was captured in a local snare baited with corn. The basisphenoid-occipital suture is fused. External measurements were made in the field: TL 256, HB 106, TV 154, HF 21, EL 20, Wt 29.

**Type Locality.** Tanzania, Rukwa Region, Sumbawanga District, Mbizi Forest Reserve, 0.5 km N, 4 km E of Wipanga, elevation 2200 m, 7.8639° S, 31.6694° E.

**Paratypes** ( $n = 32$ ). Mbizi Forest Reserve, 0.5 km N, 4 km E of Wipanga, elevation 2200 m, 171357–171361, 171363–171367, 171517–171518 (6 m, 6 f), 7.8639° S, 31.6694° E; Mbizi Forest Reserve, 0.5 km S, 3 km E of Wipanga, 2300 m, 171342–171356, 171512–171516 (12 m, 8 f), 7.8639° S, 31.6694° E.

**Diagnosis.** UTR >3.97 mm, maxillo-palatal suture located at the rear third to the rear half of  $M^1$  (Fig. 8d), rostrum elongate (LN/ONL = 37.33, LN > 9.3; Fig. 12b, Table 4), very large posterior palatal foramen (ca. 0.7–0.9 mm) starting between  $M^1$  and  $M^2$  and extending into anterior half of  $M^2$  (Figs 8d, 12d), very large sub-squamosal foramen (30–40% area of postglenoid foramen, Fig. 12f), fronto-parietal suture shallow V-shaped, zygomatic plate only slightly sinuous in lateral view, incisive foramina fall either just short of the alveoli of  $M^1$  or barely reaching alveoli of  $M^1$  (Figs 8d, 12d). T1 of  $M^1$  deflected far posteriorly (Fig. 7a).

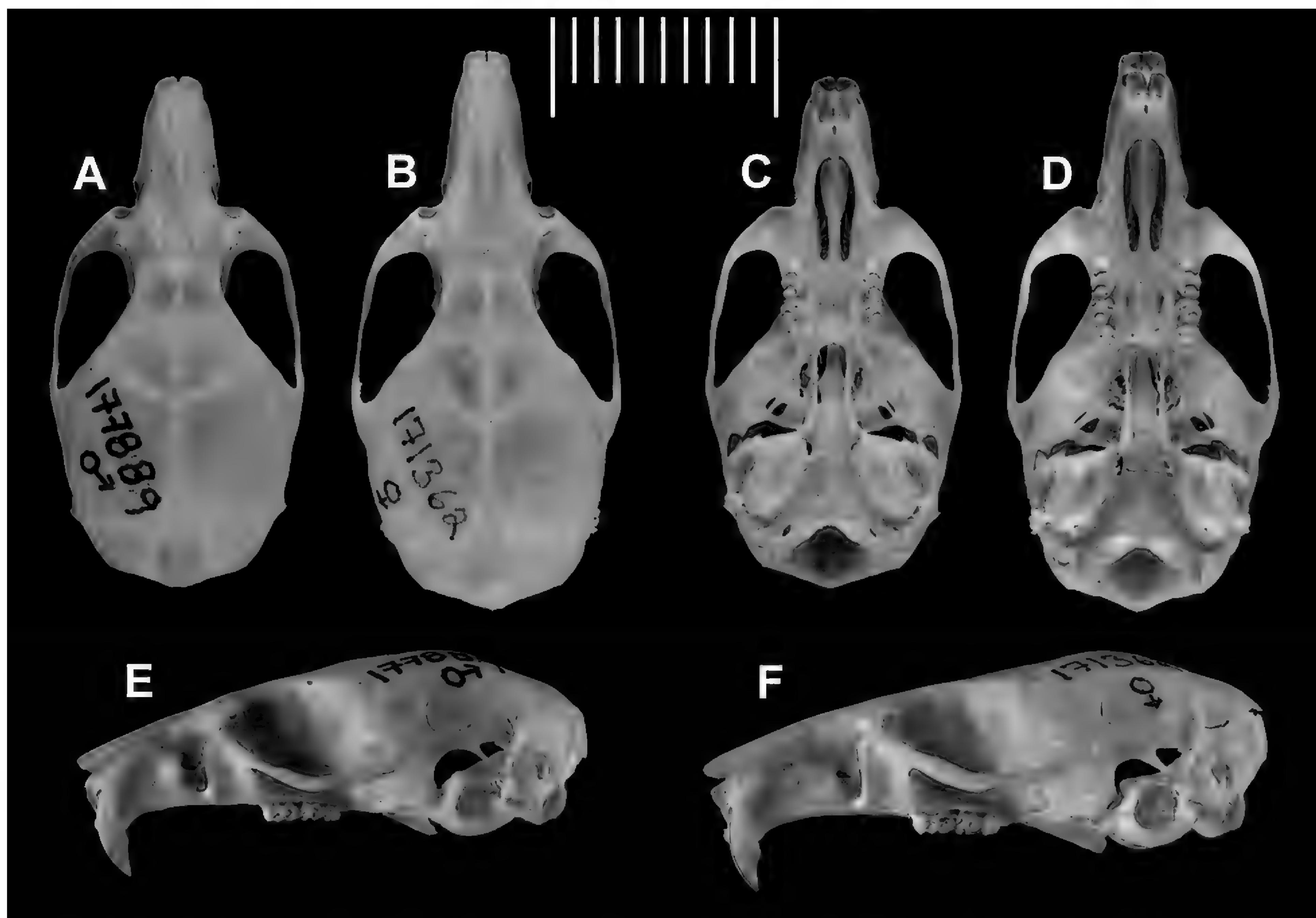


**Fig. 11.** Skins of two new species of the *Hylomyscus anselli* group from Tanzania. (a) Ventral and (c) dorsal views of *Hylomyscus mpungamachagorum* sp. nov., (b) Ventral and (d) dorsal views of *Hylomyscus stanleyi* sp. nov.

**Comparisons.** One of the largest members of the *H. anselli* group with an upper molar crown length over 4.0, thereby needing comparison with only *Hylomyscus heinrichorum* and *H. anselli*. Compared to *H. heinrichorum*, *H. stanleyi* is typically orthodont vs. slightly opistodont, see Carleton et al. 2015: fig. 7c (as *H. stanleyi*) vs. fig. 7b, has longer nasals (mean of 10.1 vs. 9.2; Ibid. 6c as *H. anselli* vs. 6b) with a more narrow zygomatic plate (Ibid. fig. 6c as *H. anselli* vs. fig. 6b, mean of 2.3 vs. 2.6) and with incisive foramina that do not penetrate the upper tooth row or alveoli (Ibid. fig. 6c as *H. anselli* vs. 6b). Since the sample size of available *H. anselli* is small ( $n = 4$ ), we expect these differences may become more

ambiguous as older individuals and additional samples of *H. anselli* become available.

**Description.** Size large (mean HB = 102, mean mass = 27.5 g; Table 5). Tail 43% longer than HB. Tail unicolor with ca. 15 annulations per cm. Belly hairs ca. 9 mm, basal 6 mm slate grey, apical 3 mm white. Dorsal hairs ca. 11 mm, basal 6 mm slate grey, terminal 3 mm tipped with light brown. Dorsum of head same color as dorsum of body. Upper lip with whitish hairs but with grey roots. Ears with blackish skin, hairs barely visible. Vibrissae long, up to 35 mm, black in color. Pes dirty white in appearance due to white hairs overlaying darker skin. Manus white. The hind foot possesses the standard



**Fig. 12.** Skulls of two new species of the *Hylomyscus anselli* group from Tanzania. (a) Dorsal, (c) ventral and (e) lateral views of *Hylomyscus mpungamachagorum* sp. nov., (b) Dorsal, (d) ventral and (f) lateral views of *Hylomyscus stanleyi* sp. nov.

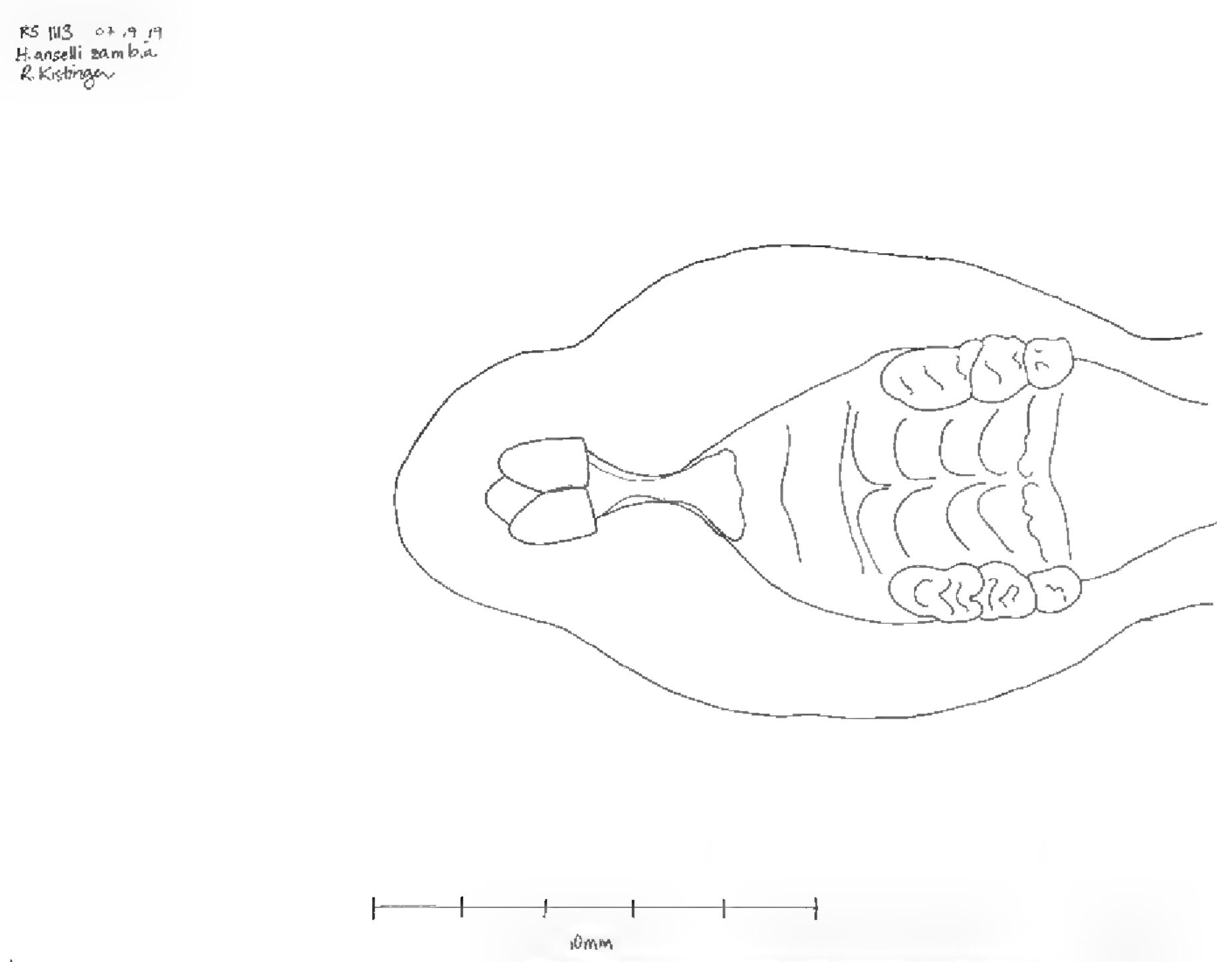
murine complement of 6 pads (see Ibe et al. 2014: fig. 2, II for reference); there is a single accessory pad on each of the 1<sup>st</sup>, 2<sup>nd</sup> and 4<sup>th</sup> interdigital pads; the first is larger and well-integrated into the 1<sup>st</sup> interdigital pad, the 2<sup>nd</sup> is small but distinct from the 2<sup>nd</sup> interdigital pad, while the 4<sup>th</sup> is smaller but distinctly separate from the 4<sup>th</sup> interdigital pad (Fig. 9c). There are seven fleshy palatal ridges: two are continuous and pre-dental, one is discontinuous and pre-dental, and the last four are discontinuous and inter-dental (Fig. 13).

Skull large (mean ONL = 27.2, mean CLM = 4.1, Table 4). Rostrum much longer than other members of the '*H. anselli*' group (LN/ONL = 37.3%), with nasals exceeding 9.3 mm (Table 4). Upper incisors generally orthodont, but a few slightly opisthodont. Incisive foramina fall short of upper tooth row crown but may reach alveoli. T3 on M<sup>2</sup> is large. Braincase elongate rather than rounded or globular. Hamular process of the squamosal thin, allowing for a large subsquamosal fenestra (ca. 30% of post glenoid foramen). Maxillo-palatal suture at third lamina of the M<sup>1</sup>. Post palatal foramina large (0.7–0.9 mm), beginning between M<sup>1</sup> and M<sup>2</sup> and continuing through the 1<sup>st</sup> third or half of the M<sup>2</sup>. Frontoparietal suture V-shaped.

Zygomatic plate narrow (mean breadth = 2.3 mm) and orthogonal to the long axis of the skull. Mesopterygoid fossa more rectangular at rostral end.

As a member of the *Hylomyscus anselli* group, the following characters are relevant: 1) mammary formula 2+4, 2) upper incisors orthodont with some individuals (5 of 33) slightly opisthodont, 3) T3 on M<sup>1</sup> is large and is more or less equal in size to T1, but T1 is deflected further posteriorly (Fig. 7a), 4) T9 on M<sup>1</sup> is distinct (Fig. 7a), 5) interorbital constriction has a weak shelf, 6) rostral length is long, LN/ONL = 37.3%, 7) incisive foramen is short, falling just short of the M<sup>1</sup> or barely meeting the beginning of the alveolus, 8) the hamular strap is thin allowing for well-developed subsquamosal foramen but which is proportionately smaller than in the two new DRC species (Figs 6e–f vs. Figs 12e–f).

**Habitat.** Mbizi is the largest area of montane cloud forest (ca. 2,000 ha) remaining on the denuded Ufipa Plateau. All of the extant forest is contained within the Mbizi Forest Reserve and is discontinuous with forest patches interspersed with grasslands. There is very little continuous canopy cover due to removal of commercially valuable



**Fig. 13.** Sketch of soft palate of *Hylomyscus anselli* from Zambia displaying the 2+(1+4) dental ridges format typical of the *Hylomyscus anselli* group: from tip of rostrum (to the left): 2 continuous pre-dental ridges, 1 discontinuous pre-dental ridge, 4 discontinuous inter-dental ridges.

timber species and exploitation for firewood and charcoal (Rodgers et al. 1984). The forest is on the eastern facing escarpment overlooking the Rukwa Trough. Surveys of the vegetation at Mbizi include those by Mtuy & Mkude (1974), Rodgers et al. (1984), and Rufo & Mabula (1987). The forest canopy reaches 25 m in places with dominant tree species being *Agarista salicifolia*, *Allophylus abyssinicus*, *Croton megalocarpus*, *Macaranga capensis*, *Neoboutonia macrocalyx*, *Olea chrysophylla*, *Olinia rochetiana*, and *Prunus africana* (Rodgers et al. 1984). The understory is between 8–20 m with the most commonly encountered tree species being: *Bersama abyssinica*, *Cathula edulis*, *Clerodendron stuhlmannii*, *Bridelia brideliifolia*, *Polyscias fulva*, and *Rhus natalensis* (Rodgers et al. 1984, Rufo & Mabula 1987). The most striking aspect of Mbizi forest is scattered *Euphorbia ampliphylla* that emerge above the canopy up to 35 m (Rodgers et al. 1984). Forest cover has been fragmented by past fires and smaller patches are now isolated on the periphery and in sheltered valleys. These are surrounded by species-rich grasslands maintained by nearly annual fires (Rufo & Mabula 1987). The central forest block is more or less continuous and covers an area of over 2000 ha. However, much of this is disturbed as the reserve is surrounded by villages and heavily exploited for firewood and charcoal. The area has many endemic plants (e.g., *Brillantaisia richardsiae*, *Glossostelma mbisiense*, *Pachycarpus pachyglossus*, *Spermacoce azurea*, *Sebaea perpava*, *Afrotysonia pilosicaulis*) and several undescribed plant species including a possible new species of *Ocotea* (Q Luke, pers. comm.).

**Reproduction.** Of the 33 examples of the new species collected (July–Aug 2001), there were 18 males and 15 females. All can be considered adult (3<sup>rd</sup> molars in advanced wear). Of the 15 females, 14 were inspected internally: 7 were pregnant, 7 were not. All pregnant females had either 4 or 5 embryos with an average crown rump length of 12–13 mm. The pregnant females averaged 28 g in weight while those that were not pregnant weighed an average of 25 g. All males were adult with scrotal testes. The entire population had well-worn molars, at least in advanced wear stage IV (Verheyen & Bracke 1966).

**Distribution.** Known only from two montane forest localities within Mbizi Forest, SW Tanzania: the type locality at ½ km S and 3 km E Wipanga, 2300 m (31.6667° E, 7.875° S) and a second locality ½ km N, 4 km E Wipanga, 2200 m (31.6694° E, 7.8639° S).

**Etymology.** Named for William T. Stanley (1957–2015) who directed the collection of all known specimens (n = 33) of this species from the Mbizi Mountains of Tanzania in 2001 as well as the type specimen of the other three species described in this manuscript (and many more). We recommend “Stanley’s wood mouse” as an English common name.

***Hylomyscus mpungamachagorum* sp. nov.** Demos, Hutterer & Kerbis Peterhans

*urn:lsid:zoobank.org:act:2831AD81-B8BF-41FC-82D3-35F2069C016A*

**Holotype.** Field Museum of Natural History, Division of Mammals, FMNH 177889 (field number WT Stanley 5988), collected by WT Stanley, 25 Aug 2003 (originally listed as *Hylomyscus* sp.). The specimen, consisting of a study skin and skull with postcranial skeleton, is an adult scrotal male (testes 13 × 8 mm) with worn first upper molar (stage IV of Verheyen & Bracke 1966). The basisphenoid-occipital suture is fused. External measurements were made in the field: TL 244, HB 99, TV 147, HF 20, EL 19, Wt 20.5. The holotype was collected in a standard snap trap (Museum Special).

**Type locality.** Tanzania, Kigoma Region, Kigoma District, Mahale Mountains, Mahale National Park 2100 m, 0.5 km NW Nkungwe Hill summit (29.77895° E, 6.10433° S).

**Paratypes** (n = 11). Tanzania, Mahale National Park, Mahale Mountains, Mahale National Park 2100 m, 0.5 km NW Nkungwe Hill summit (29.77895° E, 6.10433° S) 178011, 177888, 177890–177892, 177911; Kabezi River, 1180 m, 193233 (29.8317° E, 6.1131° S); Mahale National Park, Mfitwa Mt, 2440 m, 193234, 193218 (29.7939° E, 6.1317° S); Mahale National Park, 0.5 km S of Pasagulu Hill, 1420 m (29.75353° E, 6.06618° S), 177886, 177887.

**Diagnosis.** A member of the *H. anselli* group with a short rostrum (nasals are 34.5% of ONL, Table 4), posterior palatal foramen located posteriorly (at the first lamina of  $M^2$ , Figs 8c, 12c), and maxillo-palatal suture located posteriorly – either between second and third lamina of  $M^1$  or at level of third lamina of  $M^1$  (Figs 8c, 12c). Zygomatic plate slightly sinuous in lateral view. Sub-squamosal fenestra moderate (ca. 25–30% of postglenoid foramen; i.e., Carleton & Stanley 2005: fig. 6; Fig. 12e). Incisive foramina penetrate the alveoli of the upper tooth row (Figs 8c, 12c). Frontoparietal suture shallowly U-shaped.

**Comparisons.** A mid-sized member of the *Hylomyscus anselli* group with an upper molar crown length ca. 3.8 mm, needing comparison only with *H. kerbispeterhansi* and *H. arcimontensis*. It is unique among these three in the location of its posterior palatal foramina, located at the beginning of the second molar (Figs 8c, 12c); in *H. kerbispeterhansi* it is located at the third lamina of the  $M^1$ , while in *H. arcimontensis* it is located between  $M^1$  and  $M^2$ . The morphometrics of *H. mpungamachagorum* align closely with *H. arcimontensis*; indeed, it falls completely within its morphometric space (Figs 1–2). Compared to *H. kerbispeterhansi*, the skull is smaller (CI 23.3 vs 24.7, Table 4) and with shorter nasals (LN 8.7 vs 9.45) and diastema (LD 6.95 vs. 7.82) (Table 4).

**Description.** Size medium (mean HB = 98, CLM 3.74–3.85, mean mass = 22 g). Mean tail length 41% longer than HB. Tail unicolor with ca. 15 annulations per cm. Belly hairs 6 mm, basal 3 mm slate grey, distal 3 mm light brown. Dorsal hairs 10 mm, basal 8 mm slate grey, apical 2 mm light brown. Dorsum of head same color as back. Upper lip with white hairs, dark grey roots. Vibrissae long, black, up to 35 mm in length. Pes dirty white in appearance due to white hairs overlaying darker skin. Manus white. Ears black, hairs inconspicuous. The hind foot possesses the standard murine complement of 6 pads (see Ibe et al. 2014: fig. 2 II for reference); there is a single accessory pad on each of the 1<sup>st</sup> and 4<sup>th</sup> interdigital pads; the first is large and well-integrated into the 1<sup>st</sup> inter-digital pad, while the 4<sup>th</sup> is smaller and distinct from the 4<sup>th</sup> interdigital pad (Fig. 9b). There are seven fleshy palatal ridges: two are continuous and pre-dental, one is discontinuous and pre-dental, and the last four are discontinuous and inter-dental (as in Fig. 13).

Skull medium (mean ONL=25.25 mm, mean CLM = 3.8 mm). Rostrum moderate (nasals 34.5% GLS). Upper incisors orthodont but slightly opistodont in younger individuals. Incisive foramina just reach the  $M^1$  alveoli. T3 on  $M^2$  is tiny but distinct as in the other species described here. Braincase elongated. Hamular process of the squamosal long and thin, allowing for a moderate subsquamosal fenestra (ca. 25% of postglenoid foramen). Maxillo-palatal suture either falling between 2<sup>nd</sup> and 3<sup>rd</sup> lamina of  $M^1$  or at 3<sup>rd</sup> lamina of the  $M^1$ . Post palatal foramina

small, lying level with 1<sup>st</sup> lamina of  $M^2$ . Zygomatic plate narrow (mean = 2.3 mm) and slightly sinuous. Mesopterygoid fossa rounded at rostral end.

In comparison with other members of the *H. anselli* group, the following characters are relevant: 1) mammary formula 2+4, 2) upper incisors orthodont but younger individuals (wear stage III and IV, i.e., FMNH 177890–177892, 193218) slightly opistodont, 3) T3 on  $M^1$  is large, the anterior chevron more or less equal in size to T1 and T1 not as deflected far posteriorly as in *H. stanleyi* (Fig. 7d), 4) T9 on  $M^1$  is distinct (Fig. 7d), 5) interorbital constriction has a weak shelf, 6) rostral length (LN/ONL) is moderate at 34.5%, 7) incisive foramen meets the beginning of the  $M^1$  alveoli (in the four younger individuals the incisive foramina penetrate the upper tooth row crowns), 8) the hamular strap is long and thin and the subsquamosal foramen is well developed but proportionately smaller than in the new Congolese species (*H. pygmæus*, *H. thornesmithae*) described above (Figs 12e).

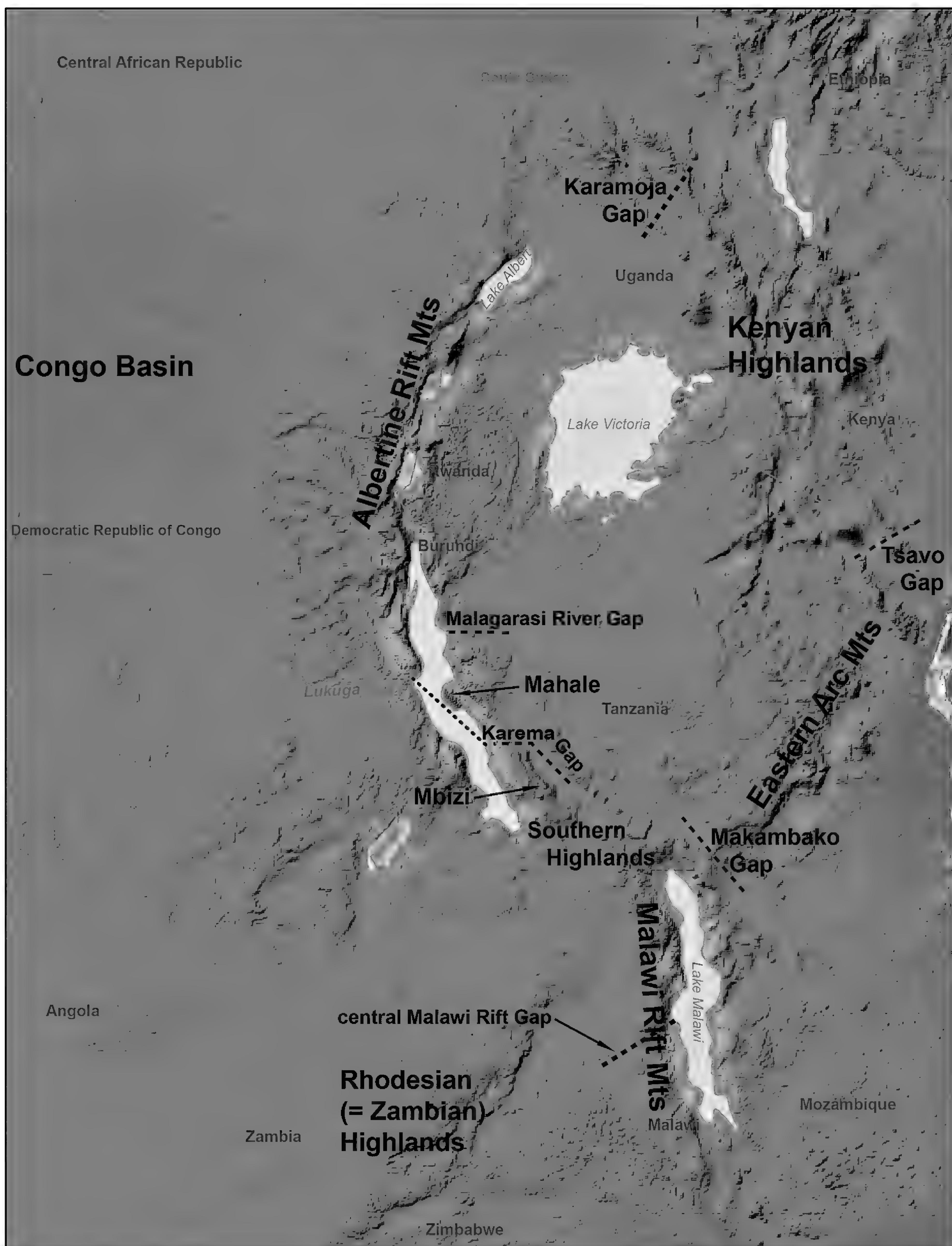
Unlike other species described herein, several skulls of this taxon show anomalous patterns of the palate including extra post palatal foramen (FMNH 177887, 177888, 193218) and interrupted incisive foramina (FMNH 177886, 177887). One specimen (FMNH 193234) also displays a white tail tip.

**Distribution.** Known only from Mahale National Park, 1180–2440 m, western Tanzania.

**Reproduction.** Of the specimens collected, there were 7 males and 5 females. The reproductive condition of 4 females was inspected: females collected on Aug 25 & 28, 2005 had no embryos (FMNH 177888, 177890) but the former was lactating. Two others were in the early stages of pregnancy: FMNH 178011, collected on 25 Aug., 2003 (CR = 5 mm) and FMNH 193233, collected 9 Nov., 2005 (CR = 7 mm).

**Habitat.** The Mahale Peninsula is predominately covered with *Brachystegia* (Miombo) woodland, but higher elevations on the Mahale Ridge are covered in montane grasslands and forest (Itani 1990). Forest is also found on the western and southwestern slopes from the ridge at 2400 m down to lowland forests at 780 m on the shore of Lake Tanganyika. The montane forest vegetation is an outlier of the Albertine Rift forests. Over 1,170 plant species, of which 39 are Albertine Rift endemics, have been recorded at Mahale (Nishida & Uehara 1981; Nishida 1990; Plumptre et al. 2007).

One specimen was collected in riverine forest along the Kabezi River. This river and other smaller tributaries flowing down the Mahale and Kabezi ridges are lined with riverine forest surrounded by tall Miombo woodland with stands of solid-stemmed bamboo, *Oxytenanthera abyssinica*. Higher up on the Mahale Ridge, the Miombo gives way to tall montane grassland dominated



**Fig. 14.** Map of current biogeographic units and their possible barriers in eastern Africa as relevant to the *H. anselli* and *H. denniae* groups.

by *Hyparrhenia* spp., *Themeda triandra*, and *Festuca* sp., with scattered *Protea gauguieri*, *Erythrina abyssinica*, *Cussonia arborea* and isolated *Parinari curatellifolia*. The riverine forest canopy reaches 30 m in places and is dominated by *Newtonia buchananii*, *Parinari excelsa*, *Bridelia micrantha*, and *Spathodea campanulata*, with occasional *Tabernaemontana stapfiana*, *Ficus sur*, *Ficus thonningii*, *Zanha golungensis*, *Prunus africana*, and *Myrianthus holstii*. The understory is dominated by woody shrubs and *Aframomum alboviolaceum* (Plumptre et al. 2003; Moyer 2006).

Two specimens originated in the Mfitwa Forest, on the SE side of the Mfitwa Peak (6°7'54" S, 29°47'38" S, 2440 m) which is surrounded by extensive areas of species-rich montane grasslands. The dominant species included *Festuca* sp., *Themeda triandra*, and *Hyparrhenia rufa*. Mature parts of Mfitwa forest in sheltered valleys reach a canopy height of 30 m. Dominant species include: *Polyscias fulva*, *Parinari curatellifolia*, *Agaurista salicifolia*, *Croton megalobostrys*, *Croton sylvaticus*, *Bersama abyssinica*, *Trichilia emetica*, *Ficus thonningii*, *Myrianthus arboreus*, *Maesa lanceolata*, and *Syzygium caminii*. The diverse forest understory is dominated by *Olyra latifolia*, *Dracaena laxissima*, *Mondia whitei*, *Reinaria engleri*, *Psychotria* sp., dense stands of bracken fern, *Pteridium aquilinum*, in light gaps and at the forest edge. Large areas of the forest are covered in nearly impenetrable monodominant stands of montane bamboo, *Oldeania alpina*, with very few other species penetrating this heavily shaded zone. Occasionally a forest tree emerges through the canopy in such stands; these areas are likely to be secondary, and at least some of them were under cultivation in the past (Itani 1990).

**Etymology.** The species is named for Noah E. Mpunga and Sophy J. Machaga, who run the Southern Highlands Conservation Program for the Wildlife Conservation Society. These leading conservationists have dedicated the last 20 years to helping describe, advocate for, and protect some of Tanzania's most threatened and iconic species. We recommend "Mahale wood mouse" as an English common name.

## DISCUSSION

### Comments on cryptic diversity

With the results presented here, the numbers of recognized species of *Hylomyscus* have increased from 8 to 21 since 2005. The 160% increase documented here over the past 14 years for *Hylomyscus* is an extraordinary number for a continental system; typically, sites of high increase in newly described species diversity are found on island systems (e.g., Madagascar, Goodman & Soarimalala 2018; Indonesia, Demos et al. 2016; Philippines, Heaney

et al. 2016). For *Hylomyscus*, such increases are due to the resurrection of synonymized taxa, new explorative surveys, and the recognition of cryptic species through genetic methods. Recently, based on molecular results, Nicolas et al. (2020) have shown that an additional 8–10 species remain undescribed from the genus, including potentially one more from the *anselli* group (figs 1–2: H. sp7). Accordingly, we predict a further increase of at least 30% over the next decade within the genus, because other species groups, especially those primarily distributed in the Congo Basin, have not yet been satisfactorily collected, documented or analyzed. We predict comparable increases will be attained in several other African small mammal groups including *Graphiurus*, *Dendromus*, *Crocidura* and various microbat species complexes currently being documented by Demos, Patterson, Eselstyn, Voelker, Kerbis Peterhans and colleagues (e.g., Demos et al. 2018, 2019a, 2019b; Patterson et al. 2019). Such cryptic, typically nocturnal, small mammal species that form the bulk of mammalian diversity (>70%), are often unrecognized by the scientific community. As an alternative, the work of Krásová et al. (2019) has taken a different approach in their review of the *Mus triton* complex; despite relatively important genetic multilocus differences among allopatric populations they have not split the complex into multiple species. Genetic analyses provided here are crucial in uncovering cryptic diversity within *Hylomyscus*. Although similar morphologically, *H. stanleyi* is only distantly related to true *H. anselli*, with which it had been co-mingled. Its close geographic proximity (ca. 400 km) and similar phenetics made it tempting to include the two as a single taxon (Carleton & Stanley 2005; Carleton et al. 2006; Demos et al. 2014a; Carleton et al. 2015; Nicolas et al. 2020).

Sadly, despite the lip-service, this is an era marked by declining resources and opportunities for field work and surveys, closure of dozens of natural history collections, a collapse in the number of trained alpha taxonomists and a lack of appreciation for the importance of biodiversity and the willingness to document it (Winker 1996; Tewksbury et al. 2014). Further, some conservation administrators feel it more important to protect individual organisms rather than entire ecosystems (Goodman & Lanyon 1994), a short-sighted strategy easily maintained by arm-chair 'conservationists'. Although this strategy may make sense among high profile taxa like gorillas or pandas, many ecosystems do not boast such charismatic species. The high fecundity of most small mammal species makes them quite resilient to collecting, but habitat loss reduces viable populations permanently. Further, integrative taxonomic revisions of small mammals can identify the regions with the highest (most valuable) evolutionary diversity and therefore provide straightforward suggestions for prioritization of conservation efforts.

## Comments on biogeography

The *Cytb* data (Fig. 4) suggest that the once united clades of *H. anselli* and *H. denniae* (under *Hylomyscus denniae*) are not only distinct, reciprocally monophyletic, and not each other's sisters, but also have separate lowland forest origins. This answers the question posed by Carleton et al. (2006: 318) on whether or not these two montane species groups shared a recent common ancestor, relative to taxa from the Guineo-Congolian lowlands. The occurrence of two new members of the *H. anselli* group in the heart of the Congo Basin, including the first one to diverge, suggests a lowland origin for the group. Other members of core Congo Basin taxa (i.e., *H. alleni* and *H. parvus* groups) form a clade sister to the *H. anselli* group. In addition, within the *H. anselli* group, *H. pygmaeus* from the Congo Basin is sister to all other members. *Hylomyscus thornesmithae* from the Congo Basin is sister to our East Africa clade (Kenya, Tanzania and Malawi). Additional material from the Guineo-Congolian rainforest, especially from the left bank of the Congo/Lualaba River, may shed light on these biogeographic relationships, as would ancestral area reconstruction, which was not carried out in this study.

A significant phylogeographic break, shown here between the *H. anselli/H. heinrichorum* clade and the rest of the *H. anselli* clade (excepting *H. pygmaeus* and *H. thornesmithae* but including *H. kerbispeterhansi*, *H. stanleyi*, *H. mpungamachagorum*, and *H. arcimontensis*) has been depicted by Chapin (1932: fig. 18) in his delimitation of bird distributions. Chapin distinguishes a *Rhodesian Highland District* (=Zambian, including the ranges of *H. heinrichorum* and *H. anselli*; Chapin 1932: fig. 18, zone 14; see our Fig. 14) from an *East African Highland District* (zone 13, known here as the *East African* clade), including the remaining taxa. It may be that these zones are separated by the Luangwa River Valley in northeastern Zambia as has been shown for the divide between *Praomys delectorum* of northern Malawi and *Praomys jacksoni* of NE Zambia (Ansell 1978: Map 187; Bryja et al. 2012: fig. 2c). The Luangwa River also seems to be the eastern limit in the distribution of *Hylomyscus anselli* (as '*Praomys denniae*' in Ansell 1978: Map 188; Bryja et al. 2012: fig. 2d). Further collecting on both sides of the upper Luangwa River is necessary to determine if this break holds for other small mammals. This biogeographic break, as well as additional potential barriers to extant small mammal distributions, are depicted in Fig. 14. Although not as strongly supported, the sister relationship of *H. arcimontensis* to other members of the *East African* clade (Fig. 4), demonstrates the long-term isolation of the Eastern Arc Mountains from adjacent East African montane systems.

Mbizi Forest is, by definition, an Albertine Rift Forest, as it is adjacent to the SW end of Lake Tanganyika. It also lies ca. 200 km NW of the Southern Highlands of

Tanzania and floristically is most closely related to these latter forests and the mountains around the northern end of Lake Malawi (Kerfoot 1964; White et al. 2001). There is no clear geographical barrier to montane forest species between Mbizi Forest and the forests around the northern end of Lake Malawi in the Southern Highlands. These areas are connected by high ground that could have supported montane forest in a wetter and colder period. However, there may have been a rain-shadow from the Southern Highlands that would have limited the extent of forest in this area (Moreau 1966).

Mbizi also contains elements of the forests of the Kenyan Highlands (Lovett 1990) as well as the Albertine Rift forests (30 of the 70 tree species are Albertine Rift endemics; Plumptre et al. 2007). Looking to the north, mixed affinities and disjunctions in distribution may be explained by the *Karema Gap* (Moreau, 1966), which forms an ecological barrier between Mbizi forest and the forested habitats of the Mahale Mountains (aka Kungwe Forest). This is a trough nearly 100 km wide extending from the shore of Lake Tanganyika southeast to the Rukwa Valley (further discussion below). Much of the terrain in the Karema Gap lies below 1000 m and forms a major biogeographical barrier to montane forest bird species distribution (Moreau 1966). It is apparent that this gap is also an important barrier for mammals.

Mbizi Forest is at the southern end of the postulated southwestern dispersal route for Guineo-Congolian forest species into the Eastern Arc (Lovett & Wasser 1993). However, Mbizi falls within the Lake Tanganyika climactic zone (Lovett 1990) and has notable faunistic differences. A number of neo-endemic and relictual forms of birds, reptiles, amphibians, and mammals are found in Mbizi with varying affinities to taxa in the Eastern Arc, Southern Highlands, Albertine Rift, and Guineo-Congolian forests (Vesey-Fitzgerald 1964; Moreau 1966; Britton 1980; Channing & Howell 2006; Plumptre et al. 2007). This is reflected in our phylogeny (Fig. 4) as the Mbizi population (*Hylomyscus stanleyi*) is sister to *H. kerbispeterhansi* from the Kenyan Highlands rather than the more proximate Mahale population (*H. mpungamachagorum*).

There are significant differences between the small mammal communities of Mbizi and the Southern Highlands: Mbizi is inhabited by *Praomys jacksoni* (Mizerovská et al. 2019), whereas the Southern Highlands are inhabited by the phylogenetically distant *Praomys delectorum* (Bryja et al. 2014; Sabuni et al. 2017). Though they are sister species, Mbizi houses the endemic *Lophuromys sabunii*, while Southern Highlands are inhabited by *Lophuromys machangui* (Verheyen et al. 2017; Sabuni et al. 2018). Finally, Mbizi is inhabited by *Crocidura montis* 1b, while Southern Highlands are inhabited by *Crocidura montis* 3 = 'luna' (Sabuni et al. 2018: fig. 2b). On the other hand, the dry forest species living in woodland ecotones were able to disperse from northern part of South-

ern Highlands to the northwest along Lake Tanganyika (e.g., *Mus triton*, clade D, Krásová et al. 2019; *Grammomys surdaster*, clade su4, Bryja et al. 2017).

The montane forests of the Mahale Mountains are also an outlier of the Albertine Rift forests (Plumptre et al. 2007). To their south is the Karema Gap while to their north lies the 70 km. wide *Kigoma-Malagarasi River Gap*, separating Mahale from the Burundi Highlands (Moreau 1966). In an earlier, unfortunately overlooked paper, Moreau (1943) reviewed geological evidence for the origins of the Karema Gap and pointed to an ancient west to south-east trough of a geological age exceeding that of the Lake Tanganyika basin. On the west side of Lake Tanganyika, this trough is filled by the Lukuga River, which exits the lake at Kalemie (formerly Albertville). The age of this trough may explain why *Hylomyscus stanleyi* is more closely related to *Hylomyscus kerbispeterhansi* in southern Kenya (almost 1000 km to the northeast) than to the more proximate taxon, *Hylomyscus mpungamachagorum*, less than 300 km to the north. Moreau was convinced that Karema Gap is of much greater biogeographic significance than the Malagarasi River Gap.

On their western slope, Mahale forest cover is nearly continuous from the montane forests on the ridge at 2400 m down to lowland forests at 780 m on the shore of Lake Tanganyika. Only 39 of the 1,170 plant species recorded at Mahale are Albertine Rift endemics (Nishida & Uehara 1981; Plumptre et al. 2007). The lowland forests harbor mammal species more typical of the Guineo-Congolian lowland forests (Moyer 2006), including *Pan troglodytes schweinfurthii*, *Manis gigantea*, and *Protoxerus stangeri* ssp., as well as birds (e.g., *Phyllastrepus scandens*) and reptiles (e.g., *Dendroaspis jamesoni*). At least two small mammal species from the W.T. Stanley 2003 Mahale survey have an origin in the Southern Highlands of Tanzania (including Mt. Rungwe) with extensions into the Marungu Highlands of DRC. These are *Otomys lacustris* Allen & Loveridge, 1933 and *Rhynchocyon cirnei reichardi* Reichenow, 1886: see discussions in Taylor et al. (2009), Corbet & Hanks (1968), and Rathbun (2017: fig. 3). Small mammals originating in the Albertine Rift include *Grammomys* cf. *dryas*, *Praomys jacksoni* and *Sylvisorex* aff. *ruandae*. On the other hand, both new species of Tanzanian *Hylomyscus* described here (*H. mpungamachagorum*, *H. stanleyi*) are closely related to forms from the Kenyan Highlands and the Eastern Arc montane archipelago (Fig. 4). It is likely that when the ancestor of the Eastern Afromontane clade of *H. anselli* group dispersed from the Congo Basin, the Albertine Rift Mountains were already inhabited by the *H. denniae* clade. They were able to colonize the peripheral (southern) parts of these mountains, from where they colonized the Eastern Arc Mountains up to parts of the Kenyan Highlands. In both the Kenyan and Tanzanian Highlands, the *H. anselli* and *H. denniae* groups are typi-

cally mutually exclusive; only in the Mau Forest (Kenya) are both species groups found in sympatry.

Several other breaks in the distribution of the *Hylomyscus denniae* and *Hylomyscus anselli* clades, referenced in Figure 14 are worthy of mention. One of them is the restriction of *Hylomyscus denniae* to the Ruwenzori Mountains of western Uganda and eastern DRC as first demonstrated by Huhndorf et al. (2007) and subsequently by Demos et al. (2014a). This break is reinforced by the Semliki River to the south and the Victoria Nile to the north. Surveys of the Blue Mountains to the north of the Ruwenzoris would be enlightening in this regard. The arid corridor of northeastern Uganda, here called the Karamoja Gap (Fig. 14), further isolates species of *H. denniae* (sensu strictu) from other *Hylomyscus* spp. in western Kenya (e.g., Mt Elgon and Cherangani Hills; Demos et al. 2014a). Our collections and surveys have failed to detect members of the *Hylomyscus denniae* or *Hylomyscus anselli* groups in the Imatong Mountains of southern Sudan and their foothills in northern Uganda (i.e., Agora Agu Forest Reserve); only *Hylomyscus stella* has been recovered from these forests. Butynski (in litt.) believes that the primate communities (i.e., galagos and vervets) of Agora Agu/Imatong Forests are slightly more closely allied with the Albertine Rift forests than with the Kenya Highlands and further, that the Victoria Nile, despite its young age may have been a factor in isolating the chimpanzee (*Pan troglodytes*) to the Albertine Rift (although they have older mid-Pleistocene fossils in Kenya; McBrearty & Jablonski, 2005).

Another arid corridor, the Tsavo Gap (Fig. 14), separates the northernmost population of *Hylomyscus arcimontensis* (Taita Hills in SE Kenya) from another relative of the *Hylomyscus anselli* group in the Kenya Highlands (*Hylomyscus kerbispeterhansi*) as well as the more distantly related *Hylomyscus endorobae* (Mt. Kenya, the Aberdares and Mau Escarpment). It is curious that no *Hylomyscus* spp. have persisted in the Volcanic Highlands of northern Tanzania (Mt. Kilimanjaro, Mt. Meru, Arusha, Ngorongoro Crater) despite our collecting efforts in these areas. In this regard, specimens from northern Tanzania (Tengeru, Ngorongoro) referred to *Hylomyscus anselli* by Bishop (1979) in his seminal paper, were misidentified.

The distribution of *Hylomyscus arcimontensis* (indeed the genus *Hylomyscus*) comes to an abrupt end in Mwenembwe Forest, Nyika National Park, northern Malawi. This gap, called here, the central Malawi Gap, at about 12° south, lies ‘between Nyika and Mount Ntchisi within the central highlands of Malawi’ (Kaliba 2014: 213 in his discussion of bird and mammal distributions; see his fig. 6.1). In her review of avian biogeography in Malawi, Dowsett-Lemaire (1989) pinpoints the major Malawi avian break slightly further south, at 14°.

These biogeographic factors have received little attention since Moreau’s (1966) discussion of their impacts on

bird distributions. The biotic impacts of these isolating mechanisms must be considered in future conservation management decisions. Given this faunal mosaic, the discovery of pockets of endemism, and the likelihood of discovering further unknown biodiversity, we call for new surveys of the virtually undocumented highlands of the Albertine Rift along the shores of Lake Tanganyika including the Marungu Highlands of southeastern Democratic Republic of Congo (not surveyed since 1884 by Richard Böhm; Noack 1887) and Gombe Mountain NP, well known for its chimpanzees but little else.

**Key to Afromontane *Hylomyscus* (excepting W Africa) plus all members of the *H. anselli* group**

1. Supra-orbital shelf strongly beaded ..... *H. aeta* (Thomas, 1911)
2. Supra-orbital shelf not beaded ..... 3
3. Sub-squamosal foramen tiny/absent, hamular process short & thick (*H. denniae* group) ..... 5
4. Sub-squamosal foramen large, hamular process long & thin ..... 9
5. Only found in Ruwenzori montane forests, size large ONL 25.6–27.8 ..... *H. denniae* (Thomas, 1906)
6. Found in other East African montane forests ..... 7
7. Size large, CI 26.8–28.6, Kenya only: Mt Kenya, Aberdares & Mau forests ..... *H. endorobae* (Heller, 1910)
8. Size small, CI 24–26 mm, Albertine Rift S of Ruwenzoris (not including Tanzania) ..... *H. vulcanorum* (Lönnberg & Gyldestenstolpe, 1925)
9. Teats 4+4, incisors orthodont ..... *H. stella* (Thomas, 1911)
10. Teats 2+4, incisors proodont, orthodont or weakly opistodont (*H. anselli* group) ..... 11
- 11a. Crown length of upper tooth row under 3.3 (Congo Basin, small) ..... 13
- 11b. Crown length of upper tooth row 3.3–4.0 (Afromontane, medium) ..... 15
- 11c. Crown length of upper tooth row over 4.0 (Afromontane, large) ..... 19
13. Crown length of upper tooth row under 1.8, slightly proodont ..... *H. pygmaeus* (sp. nov.)
14. Crown length of upper tooth row 3.0–3.25, orthodont ..... *H. thornesmithae* (sp. nov.)
15. Post palatal foramina at rear (3<sup>rd</sup> lamina) of M1, LD and NL longer, Table 4 (montane Kenya only) ..... *H. kerbis peterhansi* Demos et al., 2014
16. Post palatal foramina between M1 and M2 or at beginning of M2, LD and NL shorter, Table 4 ..... 17
17. Post palatal foramina between M1 and M2, incisive foramina fall just short of upper molar alveoli, upper incisors orthodont (Eastern Arc only) ..... *H. arcimontensis* Carleton & Stanley, 2005
18. Post palatal foramina at beginning of M2, incisive foramina reach upper molar alveoli, upper incisors

- typically opistodont (Mahale Mts only) ..... *H. mpungamachagorum* (sp. nov.)
19. Incisive foramina penetrate CLM, slightly opistodont (Angola only) ..... *H. heinrichorum* Carleton et al., 2015
20. Incisive foramina do not penetrate CLM ..... 21
21. Occipito-nasal length under 26.4, nasal length under 9.4 (Zambia only) ..... *H. anselli* (Bishop, 1979)
22. Occipito-nasal length over 26.3, nasal length over 9.3 (Mbizi Mts only) ..... *H. stanleyi* (sp. nov.)

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## APPENDIX 1

Specimens examined in this study (n = 199). Specimens included in the morphometric analyses only are in standard type, those included in both morphometric and molecular analyses are in boldface type, and those included in the molecular analyses only are indicated with an asterisk (\*). All specimens include Field Museum of Natural History (FMNH) catalogue numbers except for the *Hylomyscus anselli* type series from the British Museum of Natural History (BMNH), National Museum of Zambia (NMZ), Specimens from the Czech Republic located at the University of South Bohemia and at the Institute of Vertebrate Biology have the following acronyms: ANG for specimens from Angola and RS for specimens from Zambia.

*Hylomyscus aeta* (1): **Uganda**, Nteko Parish, edge of Bwindi-Impenetrable NP, 1600 m: 160492\*.

*Hylomyscus anselli* (19): **Zambia**, Mwinilunga Dist.: Jimbe Stream, BMNH 74.250 (TYPE), NMZ 3639, NMZ 3808; Kasombu Stream ('Isombu', see Ansell,

1978), NMZ 3631; Nyanjowe Stream ('Nyansowe', Ansell 1978), BMNH 74.251, NMZ 3638; Sakeji Stream (Sabeji, Sekezhi; see Ansell, 1978), BMNH 61.944; Mpika Dist., Danger Hill, Lubikila Stream ('Lubilikila', 'Luitikila'; see Ansell 1978) BMNH 73.142, NMZ 2764–2767, NMZ 2769–2781; Kasanka National Park, pontoon, RS 1113–1115; Kasanka National Park, Fibwe, RS 1606–1607; Zambezi source, RS 803, 810, 811; Kifubwa Rock Shelter Stream, RS 818; Nchila Wildlife Reserve, RS 793.

*Hylomyscus arcimontensis* (58): **Malawi**, Misuku Hills, Mughese Forest, 1625 m: 196303, 196311\*, **196753**, 196754–196759, 196761; Misuku Hills, Mughese Forest, 1890 m: 196762–196769; Nyika National Park, Mwenembwe Forest, 2233m: 211576\*, 211577\*; **Tanzania**, Mt Rungwe, Rungwe FR, 5 km E Ilolo, 1870 m: 163584–163588, 163590–163595; Mt Rungwe, Rungwe FR, 6 km E, 1.2 km N of Ilolo, 2140 m: Mt Rungwe, Rungwe FR, 7 km E, 2.5 km N of Ilolo, 2410 m: 163598–163600; East Usambara Mts, 4.5 km ESE Amani, Monga Tea Estate, 870–900 m: 150120, 150121, 150123, 150124, 150125, 150142, 150143\*, 150146, 150147, 150149, 150150,

150153, 150430; East Usambara Mts, 4.5 km WNW Amani, Monga Tea Estate, 870–900 m, 150119, 151251; East Usambara Mts, 4.5 km NW Amani, Monga Tea Estate, 1100 m, 147291, 147476\*; East Usambara Mts, 6 km NW Amani, Monga Tea Estate, 1100 m, 150118\*; West Usambara Mts., 12.5 km NW Korogwe, Ambangulu Tea Estate, 1300 m, 150130, 150135, 150136, 150138, 150154, 150156, 150159.

*Hylomyscus denniae* (1): **Uganda**, Rwenzori Mts NP, Mubuku R, rt bank, Nyabitaba Hut, 2667 m: 144526\*.

*Hylomyscus thornesmithae* (5): **Democratic Republic of Congo**, 14 km N of Boende, Quartorze, 326 m: 222524 (TYPE); 4 km N of Boende, Baliko, 358 m, 2119611-219613, 219689.

*Hylomyscus endorobae* (1): **Kenya**, Aberdare Range, 3.8 km W & 2.5 km S of Gatarakwa, 2700 m: 190467\*.

*Hylomyscus heinrichorum* (25): **Angola**, Mt Moco, 83793, 83795, 83796 (TYPE), 83797, 83799, 83801–83807, 83895; Mt Soque, 83783–83792; Namba Village, ANG 210, ANG 215, ANG 237, ANG 252, ANG 259.

*Hylomyscus kerbispeterhansi* (55): **Kenya**, Cherangani Hills, Kipkunnur Forest, 2740 m: 217377, 217381–217382, 217383\*, 217384, 217385\*, 217386, 217390, 217394–217395, 217408, 217422, 217605–217610, 217612–217614; Kapenguria, 153250, 2100 m; Mau Forest, 15.5 km N, 16.4 km E Bonet, 2350 m: 209997,

210000, 210001\*, 210017 (TYPE), 210018, 210023, 210042; Mau Forest, 8.5 km N & 18.4 km E of Kericho, 2320 m: 210061–210063, 210065, 210069, 210071, Mt Elgon National Reserve, nr. Kimothon Gate, 2530 m: 217325\*, 217327–217333, 217340–217342, 217345, 217354, 217358, 217597–217600, 217601, 217602, 217604.

*Hylomyscus mpungamachagorum* (6): **Tanzania**, Mahale Mts NP, Mahale Mts, 0.5 km NW of Nkungwe Summit, 2100 m: 177911, 177888, 177889 (TYPE), 177890; Mahale Mts NP, Mahale Mts, 0.5 km S of Pasagulu Hill, 1420 m: 177886–177887.

*Hylomyscus pygmaeus* (1): **Democratic Republic of Congo**, Baleko, 358 m, 219684 (TYPE).

*Hylomyscus stanleyi* (25): **Tanzania**, Mbizi Forest Reserve, 0.5 km N, 4 km E of Wipanga, 2200 m: 171357–171359, 1171360, 171361, 171362 (TYPE), 171363, 171364–171367; Mbizi Forest Reserve, 0.5 km S, 3 km E of Wipanga, 2300 m: 171343, 171344, 171346–171348, 171350, 171351, 171352, 171353, 171354–171356, 171512, 171513.

*Hylomyscus stella* (1): **Uganda**, Bwindi-Impenetrable NP, Buhoma, 1500 m: 160511\*.

*Hylomyscus vulcanorum* (1): **Democratic Republic of Congo**, Itombwe Forest, 1.5 km S Lusasa, 2050 m: 203881\*.

## APPENDIX 2

List of GenBank sequences.

Taxon	Voucher No.	GenBank No.	Country
<i>Hylomyscus aeta</i>	FMNH 160492	MN857618	Uganda
<i>Hylomyscus allenii</i>		AF518328	Gabon
<i>Hylomyscus anselli</i>	RS1113	JX126613	Zambia
<i>Hylomyscus anselli</i>	RS1114	JX126614	Zambia
<i>Hylomyscus anselli</i>	RS1115	JX126615	Zambia
<i>Hylomyscus anselli</i>	RS1606	JX126616	Zambia
<i>Hylomyscus anselli</i>	RS810	JX126617	Zambia
<i>Hylomyscus anselli</i>	RS811	JX126618	Zambia
<i>Hylomyscus anselli</i>	RS818	JX126619	Zambia
<i>Hylomyscus anselli</i>	RS793	JX126620	Zambia
<i>Hylomyscus anselli</i>	RS803	JX126621	Zambia
<i>Hylomyscus arcimontenisis</i>	FMNH 196753	KF876468	Malawi
<i>Hylomyscus arcimontenisis</i>	FMNH 196311	KF876469	Malawi

Taxon	Voucher No.	GenBank No.	Country
<i>Hylomyscus arcimontensis</i>	FMNH 211577	KF876477	Malawi
<i>Hylomyscus arcimontensis</i>	FMNH 147476	KF810191	Tanzania
<i>Hylomyscus arcimontensis</i>	FMNH 150118	KF810192	Tanzania
<i>Hylomyscus arcimontensis</i>	FMNH 150143	KF810193	Tanzania
<i>Hylomyscus baeri</i>		JQ735509	Guinea
<i>Hylomyscus denniae</i>	FMNH 144526	KF876479	Uganda
<i>Hylomyscus endorobae</i>	FMNH 190467	KF810158	Kenya
<i>Hylomyscus grandis</i>		JQ735513	Cameroon
<i>Hylomyscus heinrichorum</i>	ANG0210	MN857622	Angola
<i>Hylomyscus heinrichorum</i>	ANG0215	MN857623	Angola
<i>Hylomyscus heinrichorum</i>	ANG0237	MN857624	Angola
<i>Hylomyscus heinrichorum</i>	ANG0259	MN857625	Angola
<i>Hylomyscus kerbispeterhansi</i>	FMNH 217383	KF810205	Kenya
<i>Hylomyscus kerbispeterhansi</i>	FMNH 217384	KF810206	Kenya
<i>Hylomyscus kerbispeterhansi</i>	FMNH 217385	KF810203	Kenya
<i>Hylomyscus kerbispeterhansi</i>	FMNH 217325	KF810239	Kenya
<i>Hylomyscus kerbispeterhansi</i>	FMNH 217358	KF810200	Kenya
<i>Hylomyscus kerbispeterhansi</i>	FMNH 217601	KF810240	Kenya
<i>Hylomyscus kerbispeterhansi</i>	FMNH 210000	KF810226	Kenya
<i>Hylomyscus kerbispeterhansi</i>	FMNH 210001	KF810231	Kenya
<i>Hylomyscus stanleyi</i>	FMNH 171352	MN857627	Tanzania
<i>Hylomyscus stanleyi</i>	FMNH 171353	MN857628	Tanzania
<i>Hylomyscus stanleyi</i>	FMNH 171360	MN857629	Tanzania
<i>Hylomyscus stanleyi</i>	FMNH 171362	MN857630	Tanzania
<i>Hylomyscus stanleyi</i>	FMNH 171363	MN857631	Tanzania
<i>Hylomyscus pamfi</i>		JQ735527	Benin
<i>Hylomyscus parvus</i>		JQ735555	Cameroon
<i>Hylomyscus pygmaeus</i>	FMNH 219684	MN857626	DR of Congo
<i>Hylomyscus simus</i>		JQ735557	Ivory Coast
<i>Hylomyscus mpungamachagorum</i>	FMNH 177888	MN857619	Tanzania
<i>Hylomyscus mpungamachagorum</i>	FMNH 177889	MN857620	Tanzania
<i>Hylomyscus mpungamachagorum</i>	FMNH 177890	MN857621	Tanzania
<i>Hylomyscus stella</i>	FMNH160511	MN857632	Uganda
<i>Hylomyscus thornesmithae</i>	FMNH 219611	MN857633	DR of Congo
<i>Hylomyscus thornesmithae</i>	FMNH 219612	MN857634	DR of Congo
<i>Hylomyscus thornesmithae</i>	FMNH 219613	MN857635	DR of Congo
<i>Hylomyscus thornesmithae</i>	FMNH 222524	MN857636	DR of Congo
<i>Hylomyscus thornesmithae</i>	FMNH 219689	MN857637	DR of Congo
<i>Hylomyscus vulcanorum</i>	FMNH 203881	KF810176	DR of Congo
<i>Hylomyscus walterverheyeni</i>		JQ735614	Central African Republic

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## Research article

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# Squamate reptiles from seasonal semi-deciduous forest remnants in southwestern Bahia, Brazil

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<sup>5</sup>urn:lsid:zoobank.org:author:A83A9207-39BF-4020-A035-92CA78381A71

<sup>6</sup>urn:lsid:zoobank.org:author:C062E3CB-D966-441A-8B77-1F94DC85FA92

**Abstract.** We present a list of Squamata from Serra do Mandim and Serra Azul, both in the Atlantic Forest domain of Southern Bahia, Brazil. We recorded 27 species (21 snakes and six lizards). Most species can be characterized as generalists with a wide distribution as *Phyllopezus pollicaris*, *Salvator merianae*, *Corallus hortulanus*, *Philodryas olfersii*, *Oxyrhopus trigeminus* and *Pseudoboa nigra*. However, some of the species are considered as being difficult to sample and restricted to forest fragments such as *Bothrops bilineatus*, *Dipsas sazimai* and *Echinanthera cephalostriata*. The snake fauna of both areas represents 70% of the species previously known for the semi-deciduous forests of the state of Bahia. Although the study region is under severe anthropogenic pressure, especially due to the expansion of livestock areas, some forest remnants still withstand a rich reptile diversity.

**Key words.** Snakes, lizards, richness, biodiversity, species distribution.

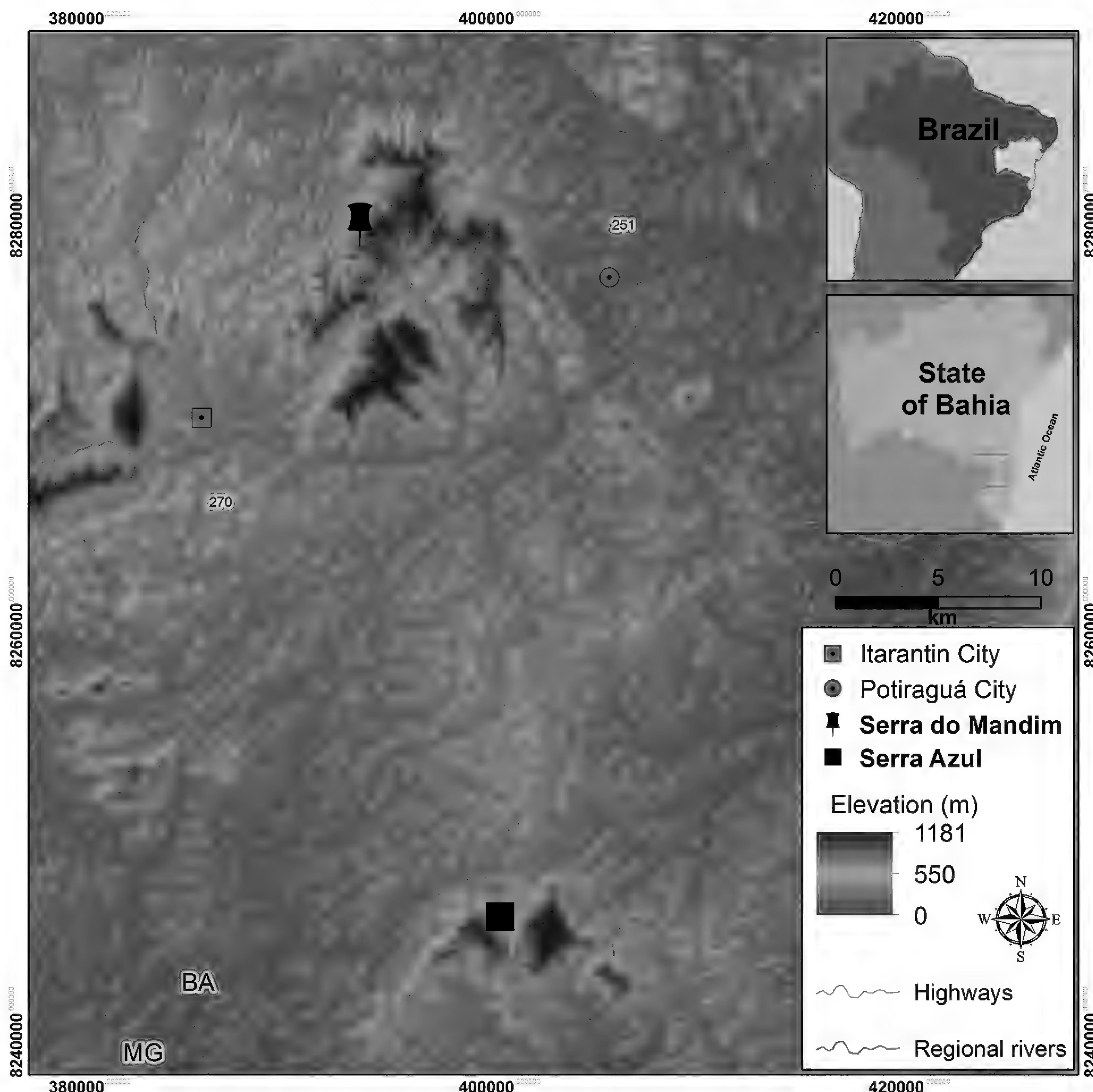
## INTRODUCTION

Habitat loss and fragmentation are considered serious threats for terrestrial reptiles (Böhm et al. 2013). Low dispersion capacity, small home ranges and a low tolerance to temperature variations turn this group, especially forest species, susceptible to changes in their natural environments (Gibbons et al. 2000). A recent study on the conservation status of the world's reptiles showed that 20% of the species are in some kind of threat category, while another 20% are classified as data deficient (Böhm et al. 2013). These authors also state that in tropical environments the number of threatened reptile species has rapidly increased due to ongoing accelerated habitat destruction.

Despite Brazil harboring one of the largest reptile diversities on the planet (Uetz et al. 1995; Costa & Bérnilds 2018), a lack of information for a large part of this tax-

onomical group is the common share, mainly concerning natural history aspects and distribution patterns (Rodrigues 2005). While a growing number of taxonomical studies have recently led to the description of several new species (e.g., Recoder et al. 2014; Fernandes & Hamdan 2014; Hamdan & Fernandes 2015; Barbo et al. 2016; Rodrigues et al. 2017; Silveira & Santos-Jr 2018; Silva et al. 2018), there is still a long way to go before this rich reptile diversity is fully understood. The assessment of the conservation status of 732 species and sub-species showed that 13% of the species must be considered as threatened or nearly threatened, while nearly 9% were characterized as “data deficient” species (ICMBio 2014).

Knowledge of the conservation status of species is essential for the implementation of conservation actions in order to mitigate the effects of anthropic actions on endangered species. In Bahia, the number of studies on reptiles has increased recently and information is now



**Fig. 1.** Studied area in the Serra do Mandim (black clamp) in the municipality of Itarantim and in the Serra Azul (black square) in the municipality of Potiraguá, in southwestern Bahia, Brazil.

available for reptiles inhabiting the central (Freitas et al. 2012), western (Freitas et al. 2016a), northern (Freitas 2014; Freitas et al. 2016b; Marques et al. 2016; Freitas et al. 2018; Freitas et al. 2019) and southern (Dixo 2001; Argôlo 2004; Dias et al. 2014) regions of the state, while the southwestern part of the state still shows a large gap concerning information on its reptiles.

The Mandim and Azul Mountains are located in southwestern Bahia - Brazil, in the municipalities of Itarantim and Potiraguá, in a region classified as semi-deciduous seasonal forest (Ibge 1997; Salino et al. 2006). They are

within the boundaries of the Rio Pardo and Rio Jequitinhonha basins, bordering the northeast of Minas Gerais. While they belong to the Atlantic Forest domain, they suffer great influence by the Caatinga and Cerrado domains. A project entitled “Biodiversity and conservation in the Jequitinhonha and Mucuri valleys” was carried out in the region, which, through biological inventories, showed that even suffering high levels of degradation, the region still maintains a great amphibian, bird, and mammal diversity (Pinto & Bede 2006). The same authors identified several priority areas for conservation



**Fig. 2.** Study areas in the southwestern region of Bahia. Fugima farm in the Serra do Mandim (A, C and E). A: Semi-deciduous forest fragment; C: stream; E: permanent pond. Serra Azul farm in the Serra Azul (B, D and F). B: Semi-deciduous forest fragment; D: stream; F: permanent pond.

and emphasized the importance of more research aimed at other still under sampled groups, as, for example, reptiles. Therefore, the objective of our study was to inventory squamate reptile species from the Serra Azul and

Serra do Mandim, aiming to fill a gap in the knowledge of the group for the state.

## MATERIAL AND METHODS

Between January 2015 and March 2016 six field campaigns were undertaken to sample squamate reptiles in two Atlantic Forest areas characterized as seasonal semi-deciduous forest in southwestern Bahia: at the “Serra do Mandim” ( $15^{\circ}37'58''$  S,  $39^{\circ}59'01''$  W) in the municipality of Itarantim, and at the “Serra Azul” ( $15^{\circ}52'01''$  S,  $39^{\circ}55'54''$  W) in the municipality of Potiraguá (Fig. 1). During every campaign each area was sampled between three and four days and nights, resulting in a total sampling effort of 44 field days by two researchers.

The climate of the region corresponds to the “Am” type of Köppen (1936), with average rainfall of 800 to 1100 mm and a temperature range between  $23.5^{\circ}\text{C}$  and  $25^{\circ}\text{C}$  (Ibge 1997). The mountains have gradients of 300–800 m of altitude.

Reptiles were sampled through active search (Rödel & Ernst 2004) at 14 sampling sites in each area, including 12 transects with 50 m length within the forest, a 120 m transect along a stream and a permanent pond (Fig. 2). All available microhabitats within five meters left and right of the transects were sampled (fallen trunks, leaf litter, vegetation and burrows). Due to increased humidity and high concentration of amphibians at the ponds, these sites were potentially more prone to reveal foraging snakes. During each field expedition, forest transects were sampled for 40 minutes and streams were sampled for 90 minutes. The surroundings of the ponds were searched for 30 minutes. The total sample effort was 60 hours in each of the areas. Occasional encounters during the team’s displacement between sample points were also recorded. Additionally to nocturnal sampling squamate reptiles were also sampled during the day from 14h to 17h on a 10 km trail leading to the areas where the transects were located.

The reptiles were collected by hand or using snake hooks and transferred to cotton bags or plastic boxes. The license to capture reptiles was issued by ICMBio (number 13709). Specimens were killed with 20% benzocaine (1mg/g), fixed in 10% formalin for seven days and stored in 70% ethanol. They were further identified using original descriptions available in the literature and deposited in “Museu de Zoologia da Universidade Estadual de Santa Cruz-MZUESC” (Appendix I).

To evaluate sample efficiency, we constructed a rarefaction curve with 1000 randomizations, using the total number of registered individuals in the study area. We used abundance data per sample to extrapolate the richness through the non-parametric estimators Chao2, Jackknife 1 and 2 and Bootstrap (Magurran 1998; Gotelli & Colwell 2001). The analyses were made using the software PAST 3.07 (Paleontological Statistics Software Package for Education and Data Analysis).

## RESULTS AND DISCUSSION

During our study we recorded 27 species of Squamata, 21 belonging to snakes and six to lizards (Table 1, Figs 3–4). Among the snakes, the Dipsadidae family was represented by 12 species, followed by Colubridae with four. Regarding lizards only the family Tropiduridae was represented by two species, while the other families only had one representative. Of all recorded Squamata, none is listed in the Brazilian list of threatened taxa (ICMBio-Portaria MMA nº 444/2014 and nº 445/2014).

The comparison with the reptile fauna from surrounding areas near Serra do Mandim and Serra Azul is hampered by the absence of such kind of studies. In general, the recorded squamate fauna (n=27) can be considered larger than that of other sampled areas in the region, as some municipalities from the northeast of the state of Minas Gerais (n=11, Feio & Caramaschi 2002) and from the APA da Lagoa Encantada, between the cities of Ilhéus, Floresta Azul and Almadina (n=17, Dias et al. 2014). Other studies undertaken in Atlantic Forest areas revealed larger squamate reptile richness, as the one by Argôlo (2004) reporting 61 species of snakes from cocoa plantations in southeastern Bahia. It is worth mentioning that this study was conducted during a time span of 12 years. Hamdan & Lira-Da-Silva (2012) reported the occurrence of 30 species of snakes for the seasonal semi-deciduous Forest of the state of Bahia. We managed to report 70% of the species reported for this kind of vegetation in the state.

Most of the recorded species show a wide distribution, occurring both in the Atlantic Forest and in the Caatinga domain, such as *Phyllopezus pollicaris*, *Salvator merianae*, *Corallus hortulanus*, *Philodryas olfersii*, *Oxyrhopus trigeminus*, *Pseudoboa nigra* and *Xenodon merremii* (Vanzolini et al. 1980; Rodrigues 1986; Argôlo 2009; Hamdan & Lira-Da-Silva 2012; Marques et al. 2012c). Others are typical from Caatinga environments, such as *Tropidurus hispidus* (Vanzolini et al. 1980; Rodrigues 2003) while others are restricted to the Atlantic Forest domain such as *Dipsas sazimai* and *Echinanthera cephalostriata*. Despite having a wide distribution range in the Atlantic Forest, *D. sazimai* has only been reported thrice from the state of Bahia (Roberto et al. 2014). This species is rare and typical of forest environments and following Fernandes et al. (2010) it should be considered potentially endangered. *Echinanthera cephalostriata* can be found in the Atlantic Forest domain from Santa Catarina to southwestern Bahia state (Argôlo & Jesus 2008). In Bahia, it is considered a rare species, with occurrence associated with montane forest, above 600m altitude (Argôlo 2009). These two species (*D. sazimai* and *E. cephalostriata*) have been included in the recently launched list of threatened species of the state of Bahia (SEMA 2017) as vulnerable (VU) and endangered (EN), respectively.

**Table 1.** Richness and composition of Squamata species recorded at the Serra do Mandim and at the Serra Azul in southwestern Bahia, Brazil. Sampling method: OE-opportunistic encounter; FT-Forest transect; P-pond; ST-Stream transect.  
\*Nomenclature follows Costa & Bérnuls (2018).

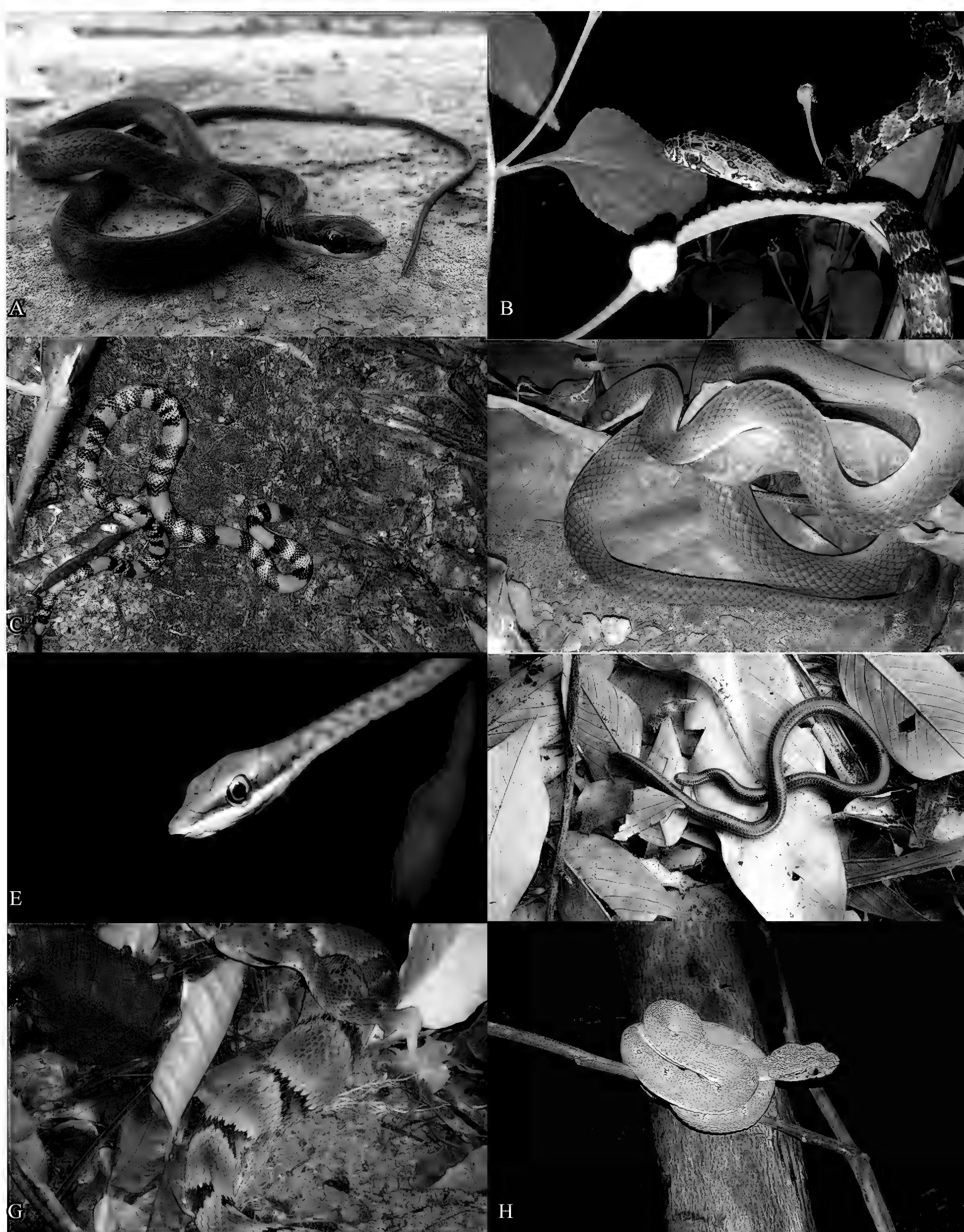
Family/species*	S. Mandim	Serra Azul	Total
<b>LIZARDS</b>			
<b>Phyllodactylidae</b>			
<i>Phyllopezus pollicaris</i> (Spix, 1825)	–	OE	01
<b>Dactyloidae</b>			
<i>Norops fuscoauratus</i> (D'Orbigny, Duméril & Bibron, 1837)	FT	–	01
<b>Leiosauridae</b>			
<i>Enyalius catenatus</i> (Wied, 1821)	FT, ST, OE	FT, OE	21
<b>Tropiduridae</b>			
<i>Tropidurus torquatus</i> (Wied, 1820)	P, OE	–	02
<i>Tropidurus hispidus</i> (Spix, 1825)	–	P	01
<b>Teiidae</b>			
<i>Salvator merianae</i> (Duméril & Bibron, 1839)	–	OE	01
<b>SNAKES</b>			
<b>Boidae</b>			
<i>Corallus hortulanus</i> (Linnaeus, 1758)	FT, ST	–	02
<b>Colubridae</b>			
<i>Chironius fuscus</i> (Linnaeus, 1758)	–	FT, ST	03
<i>Mastigodryas bifossatus</i> (Raddi, 1820)	OE	P, OE	03
<i>Oxybelis aeneus</i> (Wagler in Spix, 1824)	ST	OE	02
<i>Tantilla melanocephala</i> (Linnaeus, 1758)	–	OE	03
<b>Dipsadidae</b>			
<i>Dipsas sazimai</i> Fernandes, Marques e Argôlo, 2010	FT	FT, OE	05
<i>Echinanthera cephalostriata</i> Di-Bernardo, 1996	–	FT	02
<i>Erythrolamprus miliaris</i> (Linnaeus, 1758)	OE	P	02
<i>Erythrolamprus poecilogyrus</i> (Wied, 1825)	–	OE	03
<i>Imantodes cenchoa</i> (Linnaeus, 1758)	–	ST, OE	03
<i>Oxyrhopus petolarius</i> (Linnaeus, 1758)	–	FT	02
<i>Oxyrhopus trigeminus</i> Duméril, Bibron e Duméril, 1854	OE	P, OE	04
<i>Philodryas olfersii</i> (Liechtenstein, 1823)	OE	–	01
<i>Pseudoboa nigra</i> (Duméril, Bibrón & Duméril, 1854)	–	OE	01
<i>Sibynomorphus neuwiedi</i> (Thering, 1911)	FT	FT	02
<i>Thamnodynastes nattereri</i> (Mikan, 1828)	ST, OE	FT	04
<i>Xenodon merremii</i> (Wagler in Spix, 1824)	OE	OE	05
<b>Leptotyphlopidae</b>			
<i>Trilepida salgueiroi</i> (Amaral, 1955)	–	OE	01
<b>Viperidae</b>			
<i>Bothrops jararaca</i> (Wied, 1824)	FT, ST	FT, ST, OE	18
<i>Bothrops leucurus</i> Wagler in Spix, 1824	ST, P, OE	P, OE	07
<i>Bothrops bilineatus</i> (Wied-Neuwied, 1821)	ST	–	02
<b>Total</b>	<b>16</b>	<b>22</b>	<b>102</b>



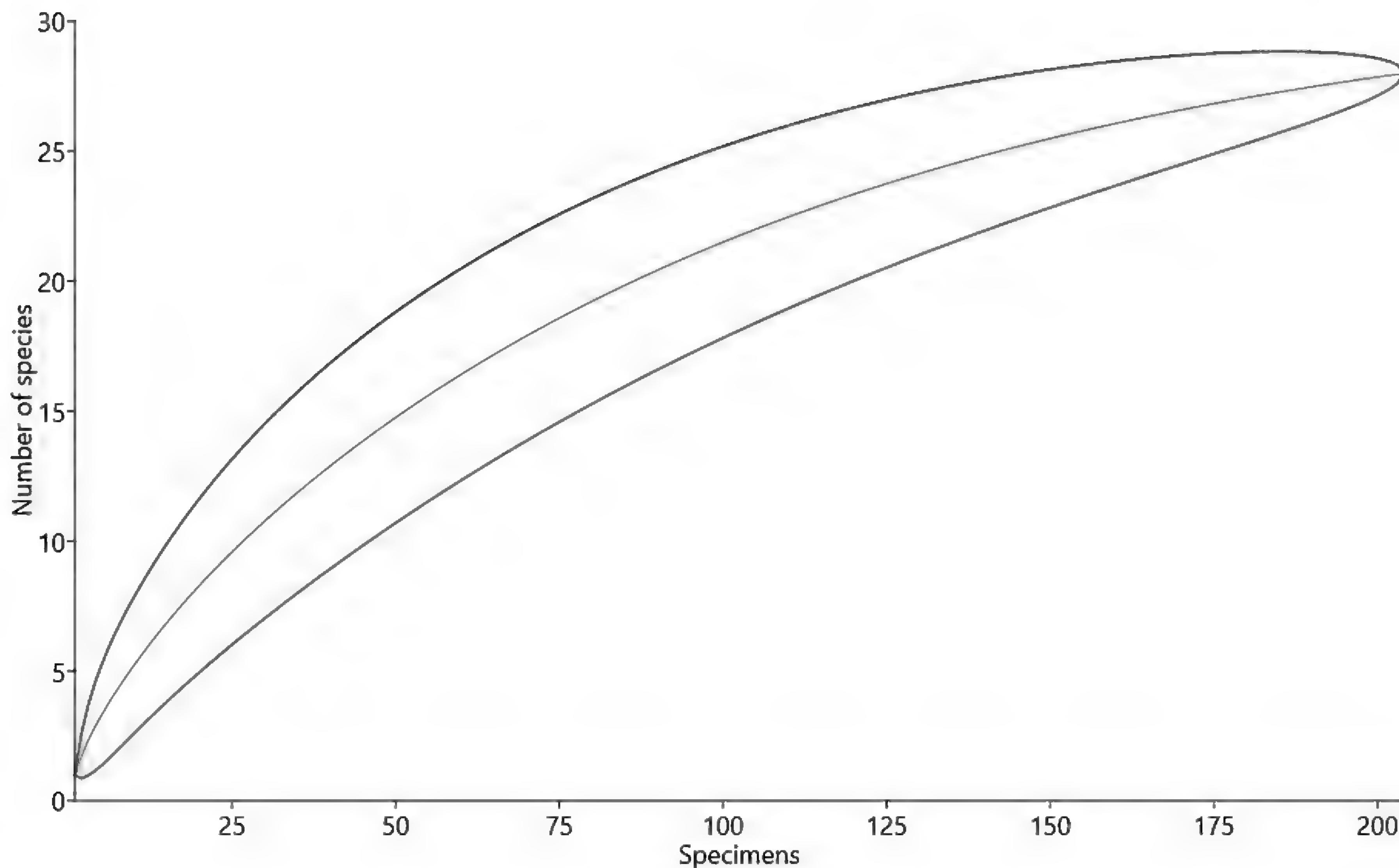
**Fig. 3.** Squamata recorded at Serra do Mandim and Serra Azul in southwestern Bahia, Brazil. **A.** *Norops fuscoauratus*; **B.** *Enyalius catenatus* (male); **C.** *E. catenatus* (juvenile); **D.** *Chironius fuscus*; **E.** *Dipsas sazimai*; **F.** *Imantodes cenchoa*.

The rarefaction curve did not reach the asymptote and remained in ascending function (Fig. 5), even though 69% to 86% of the species indicated by the richness estimators ( $\text{Chao 2} = 32.1 \pm 4.3$ ;  $\text{Jackknife 1} = 36.2 \pm 4.2$ ;  $\text{Jackknife 2} = 39.2$  and  $\text{Bootstrap} = 31.4$ ) were sampled. However, the use of additional sampling methods as pit-

falls (Cechin & Martins 2000) and funnel traps (Greenberg et al. 1994) could lead to an increase in the diversity of sampled Squamata for the region, since they allow the record of species that have specific habits and are hardly sampled during active search, such as fossorial snakes and lizards (Macedo et al. 2008).



**Fig. 4.** Squamata recorded at Serra do Mandim and Serra Azul in southwestern Bahia, Brazil. **A.** *Echinanthera cephalostriata*; **B.** *Mastigodryas bifossatus*; **C.** *Oxyrhopus trigeminus*; **D.** *O. petolarius*; **E.** *Oxybelis aeneus*; **F.** *Trilepida salgueiroi*; **G.** *Bothrops jararaca*; **H.** *B. bilineatus*.



**Fig. 5.** Rarefaction curve based on individuals of Squamata for two regions of semi-deciduous seasonal forest in Serra do Mandim and Serra Azul in southwestern Bahia, Brazil. The center line corresponds to the mean obtained with 1000 randomizations, and the lines above and below correspond to the associated standard deviation.

The present study contributes to fill a gap in the knowledge of Squamata of the southwestern region of Bahia, presenting a list with 27 species of snakes and lizards for the Serra do Mandim and Serra Azul. Although the region is suffering great anthropic pressure, mainly related to the agricultural expansion, the forest remnants still have conditions to support and maintain species considered difficult to sample in the Atlantic Forest, such as *Dipsas sazimai* and *Echinanthera cephalostriata*.

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## APPENDIX I.

List of vouchers deposited in the Museu de Zoologia da Universidade Estadual de Santa Cruz-MZUESC.

### LIZARDS

PHYLLODACTYLIDAE. *Phyllopezus pollicaris*: MZUESC 15911. DACTYLOIDAE. *Norops fuscoauratus*: MZUESC 15931. LEIOSAURIDAE. *Enyalius catenatus*: MZUESC 15901-15906, 15916, 15917, 15920, 15921, 15922, 15930. TROPIDURIDAE. *Tropidurus torquatus*: MZUESC 15939, 15942. *Tropidurus hispidus*: MZUESC 16546.

### SNAKES

BOIDAE. *Corallus hortulanus*: MZUESC 15929. COLUBRIDAE. *Chironius fuscus*: MZUESC 15934, 15935. *Mastigodryas bifossatus*: MZUESC 16541. *Oxybelis aeneus*: MZUESC 15910, 15919. *Tantilla melanocephala*: MZUESC 15924, 15943, 16538. DIPSADIDAE.

*Dipsas sazimai*: MZUESC 15908, 15913, 15926, 16548. *Echinanthera cephalostriata*: MZUESC 15909, 15928. *Erythrolamprus miliaris*: MZUESC 15907, 15940. *Erythrolamprus poecilogyrus*: MZUESC 15944, 16540, 16543. *Imantodes cenchoa*: MZUESC 15914. *Oxyrhopus petolarius*: MZUESC 15945, 16544. *Oxyrhopus trigeminus*: MZUESC 14687, 15923. *Philodryas olfersi*: MZUESC 16547. *Pseudoboa nigra*: MZUESC 16537. *Sibynomorphus neuwiedi*: MZUESC 15927, 15932. *Thamnodynastes nattereri*: MZUESC 15912, 15933. *Xenodon merremii*: MZUESC 16534-16536, 16539, 16542. LEPTOTYPHLOPIDAE. *Trilepida salgueiroi*: MZUESC 16545. VIPERIDAE. *Bothrops jararaca*: MZUESC 14469, 14470, 14471, 15915, 15918, 15936, 15937. *Bothrops leucurus*: MZUESC 15925, 15938. *Bothrops bilineatus*: MZUESC 16549.

## Research article

urn:lsid:zoobank.org:pub:D8219FBF-35E6-4791-9EC5-519120C3B543

# Sexual morphs of the three native Nearctic species of the genus *Periphyllus* van der Hoeven, 1863 (Insecta: Hemiptera: Aphididae), with identification keys including introduced species

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**Abstract.** *Periphyllus* van der Hoeven, 1863 (Hemiptera: Aphididae: Chaitophorinae) is a Holarctic genus, with just three species native to Nearctic: *Periphyllus americanus* (Baker, 1917), *P. brevispinosus* Gillette & Palmer, 1930, and *P. negundinis* (Thomas, 1878). Males and oviparous females of *P. brevispinosus* and *P. negundinis* and males of *P. americanus* are described. Original keys to the identification of the known native and non-native sexual morphs of this genus, associated with maples in the Nearctic Region, are given.

**Key words.** Aphids, distribution, maple, sexuales.

## INTRODUCTION

*Periphyllus americanus* (Baker, 1917) (American Maple Aphid), *P. brevispinosus* Gillette & Palmer, 1930 (Colorado Maple Aphid) and *P. negundinis* (Thomas, 1878) (Boxelder Aphid) are the only Nearctic species within the genus *Periphyllus* van der Hoeven, 1863 (Hemiptera: Aphididae: Chaitophorinae) (Blackman & Eastop 2019). Apterous and alate viviparous females of these species were extensively collected, described and reported by entomologists throughout the United States and Canada (Gillette & Palmer 1930; Knowlton 1947; Essig & Abernathy 1952; Palmer 1952; Richards 1972). However, the sexual morphs of these species seem to be extremely rarely collected and their detailed description is still lacking (Blackman & Eastop 2019). In particular, the male of *P. americanus* was shortly described, as *P. palmerae* Knowlton, 1947, the oviparous female of *P. brevispinosus* was mentioned by Palmer (1952), whereas the brief description of sexuales of *P. negundinis* was provided by Essig and Abernathy (1952).

In the present study, based on the specimens deposited in the Natural History Museum, London, UK, we re-describe or describe all known sexuales of Nearctic species of the genus *Periphyllus*, except the oviparous female of *P. americanus*, which remains unknown. In addition to

these three native species, three non-indigenous species of the genus *Periphyllus* are also distributed in North America: *P. californiensis* (Shinji, 1917), *P. lyropictus* (Kessler, 1886), and *P. testudinaceus* (Fernie, 1852). According to Foottit et al. (2006), although *P. aceris* (Linnaeus, 1761) has been recorded by numerous authors as introduced to North America, it seems that those records refer to other species. As *Periphyllus* is a highly polymorphic genus, we provide original keys to differentiate all known sexuales of native and non-native species of this genus, associated with maples in North America.

## MATERIAL AND METHODS

The specimens were examined using a Nikon Ni-U light microscope equipped with a phase contrast system. The drawings of the morphological details were done free-hand on a Nikon Ni-U light microscope using a camera lucida. In each drawing, the left side represents dorsal view and the right side represents ventral view. On the dorsal side only dorsal setae are shown and on the ventral side only ventral setae are shown.

The measurements were done according to Blackman & Eastop (2019).

### Abbreviations for morphological terms

BL	= body length (from anterior border of the head to the posterior border of anal plate)
BW	= greatest body width across middle of abdomen
ANT	= antenna or its length
ANT I–VI	= antennal segments I–VI or their lengths (ratios between antennal segments are simply given as e.g., 'VI:III')
LS ANT III	= length of longest seta of ANT III
BD III	= basal articular diameter of ANT III
BASE	= basal part of the last antennal segment or its length
PT	= processus terminalis of the last antennal segment or its length
ARS	= apical segment of rostrum or its length
FEMORA III	= hind femora length
TIBIA III	= hind tibia length
HT II	= second segment of hind tarsus or its length
ABD I–VIII	= abdominal tergites I–VIII

### Institutional abbreviations

NHMUK	= Natural History Museum, London, UK
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### RESULTS

#### *Periphyllus americanus* (Baker, 1917)

Baker, 1917: 428

Fig. 1

This species is widely distributed in the United States and Canada on several species of maples (Blackman & Eastop 2019). Specifically, it is known in Colorado, Connecticut, Florida, Idaho, Maine, Massachusetts, New York, North Carolina, Pennsylvania, Utah, Washington and Wyoming (USA), and in British Columbia, New Brunswick, Nova Scotia and Quebec (Canada) and its recorded host plants are *Acer floridanum*, *A. glabrum*, *A. grandidentatum*, *A. pseudoplatanus*, *A. saccharinum*, *A. saccharum* (Essig & Abernathy 1952; Palmer 1952; Richards 1972; Smith & Parron 1978; Knowlton 1983).

**Material examined.** UNITED STATES, Utah, Ogden, 19 October 1958, G. F. Knowlton leg., 1 alate male.

**Alate male** (Fig. 1). Colour in life: unknown; mounted specimens with head, antennae, pronotum, sclerites, siphunculi and genitalia dark. Legs dark with basal part of femur slightly paler. Body 2.92 mm long and 1.02 mm width. Head with 4–6 pairs of long fine, pointed setae 0.16–0.22 mm long (Fig. 1a). ANT 6-segmented, 2.12–

2.15 mm long (Fig. 1b), reaching ABD VI, 0.71–0.73 × BL. ANT IV 1.50–1.56 ANT V; ANT V always shorter than ANT VI; PT 2.76–3.00 × BASE; other antennal ratios: VI:III 0.70–0.71, V:III 0.47–0.48, IV:III 0.72–0.74. ANT I with 4–8 setae, ANT II with 3–4 setae, ANT III 0.67–0.70 mm long with 11–13 setae (7–8 long pointed setae and 4–5 thick pointed setae), ANT IV 0.50–0.51 mm long with 5–6 setae (3–4 long pointed setae and 1–2 thick pointed setae), ANT V 0.32–0.34 mm long with 1 setae, BASE 0.12–0.13 mm long with 2 setae, PT 0.36 mm long with 3 apical setae. ANT setae: fine, pointed, up to 0.1 mm long; thick, pointed up to 0.025 mm long. ANT III setae 0.01–0.10 mm long. LS ANT III 2.5 × BD III. The whole ANT III–V covered by rounded secondary rhinaria: ANT III with 59–63 rhinaria, ANT IV with 18–32 rhinaria, ANT V with 8 rhinaria. Rostrum reaching hind coxae. ARS 0.13 mm long, 0.18–0.19 × ANT III and 0.76–0.81 × HT II, with 4 accessory setae (Fig. 1c). Legs with numerous, fine and pointed setae, 0.05–0.23 mm long. FEMORA III 0.90 mm long. TIBIA III 1.30 mm long with numerous short spinules distributed on distal 1/3 of tibiae. HT II 0.16–0.17 mm long. Empodial setae spatulate; first tarsal chaetotaxy 5:5:5 (Fig. 1d). Fore wings with normal venation (Fig. 1e). Abdominal tergites membranous, with large fused spinal sclerites, pleural sclerites very small, irregularly placed, marginal sclerites oval. Abdominal setae 0.07–0.22 mm long; marginal sclerites with 4–5 setae (0.1–0.2 mm long), pleural sclerites with 0–1 setae (0.11–0.17 mm long), spinal sclerites with 4–5 setae (0.07–0.22 mm long) (Fig. 1f). Siphunculi 0.17 mm long and 0.18–0.19 width, truncate, reticulated on the whole length, with developed flange and coalescent with each marginal sclerite of ABD VI (Fig. 1g). Cauda 0.08 mm long, broadly rounded, with 8 setae (4 long and 4 short setae) 0.06–0.13 mm long (Fig. 1h). Genitalia well developed, strongly sclerotized with roundish, lobate parameres, covered by numerous spine-like setae. Basal part of phallus rectangular, shortened, with numerous short spinules (Fig. 1i).

#### *Periphyllus brevispinosus* (Gillette & Palmer, 1930)

Gillette & Palmer, 1930: 546–547

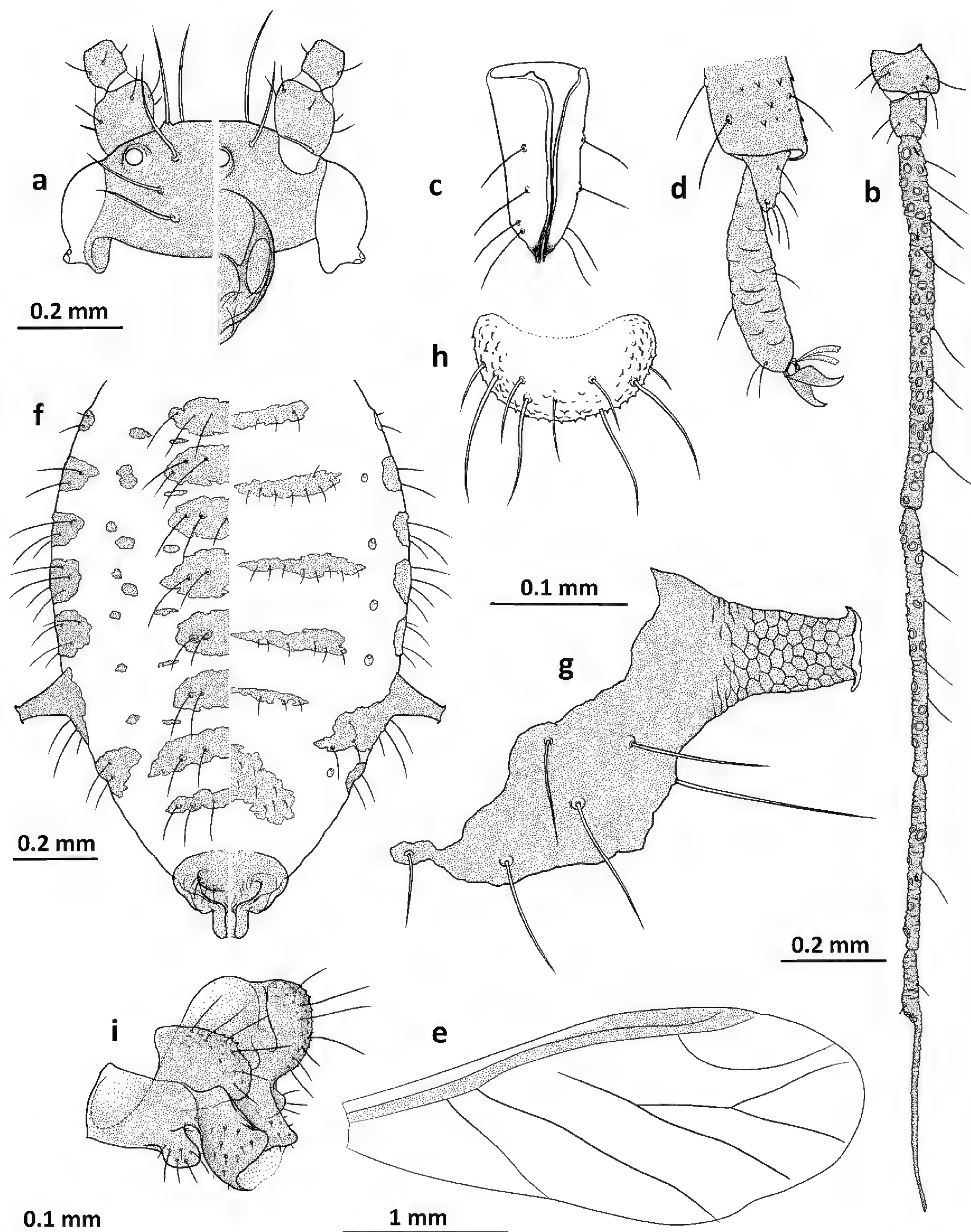
Figs 2–3

This species is known western North America on *Acer glabrum* (Blackman & Eastop 2019); specifically, it has been recorded from Colorado, Idaho, Oregon, Utah, Washington, and Wyoming (USA) and Alberta and British Columbia (Canada) (Essig & Abernathy 1952; Palmer 1952; Richards 1972; Smith & Parron 1978; Knowlton 1983).

**Material examined.** UNITED STATES, Colorado, Skyway, 15 September 1956, on *Acer glabrum*, Hottes & H.R.L. leg., 2 alate males, 4 oviparous females, BM 1984 340.

**Alate male** (Fig. 2). Colour in life: unknown; mounted specimens with head, pronotum, sclerites, siphunculi and genitalia dark. Legs light dark with basal part of femur and middle part of tibiae slightly paler. ANT I–VI dark with ANT III–IV slightly paler at base. Body 2.25–2.37 mm long and 0.80–0.87 mm width. Head with

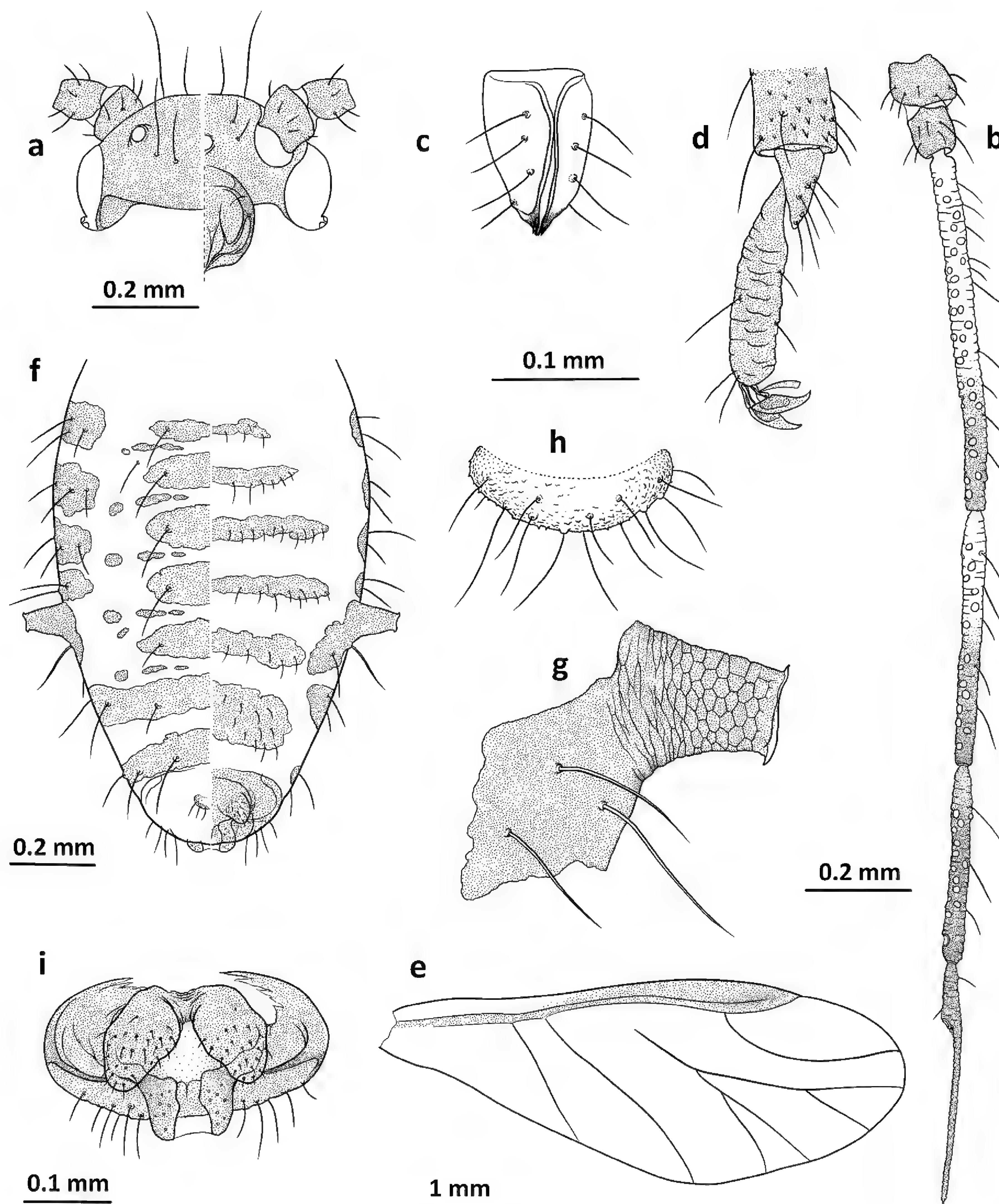
4–6 pairs of long fine, pointed setae 0.09–0.16 mm long (Fig. 2a). ANT 6-segmented, 2.00 mm long (Fig. 2b), almost reaching ABD VII, about  $0.84 \times$  BL. ANT IV 1.30–1.40 ANT V; ANT V about as long as ANT VI; PT 2.30–2.41  $\times$  BASE; other antennal ratios: VI:III 0.62–0.65, V:III 0.45–0.49, IV:III 0.65–0.69. ANT I with 7 se-



**Fig. 1.** *Periphyllus americanus* (Baker, 1917), alate male. **a.** Head. **b.** Antenna. **c.** Apical segment of rostrum. **d.** Hind tarsus. **e.** Fore wing. **f.** Abdomen. **g.** Siphunculus. **h.** Cauda. **i.** Genitalia.

tae, ANT II with 7 setae, ANT III 0.63–0.69 mm long with 7–10 setae, ANT IV 0.41–0.48 mm long with 5–7 setae, ANT V 0.31–0.34 mm long with 3–5 setae, BASE 0.12–0.13 mm long with 2 setae, PT 0.29–0.30 mm long with 3 apical setae. ANT setae fine, pointed, 0.03–0.07 mm long. LS ANT III 1.75–2.33 × BD III. The whole ANT

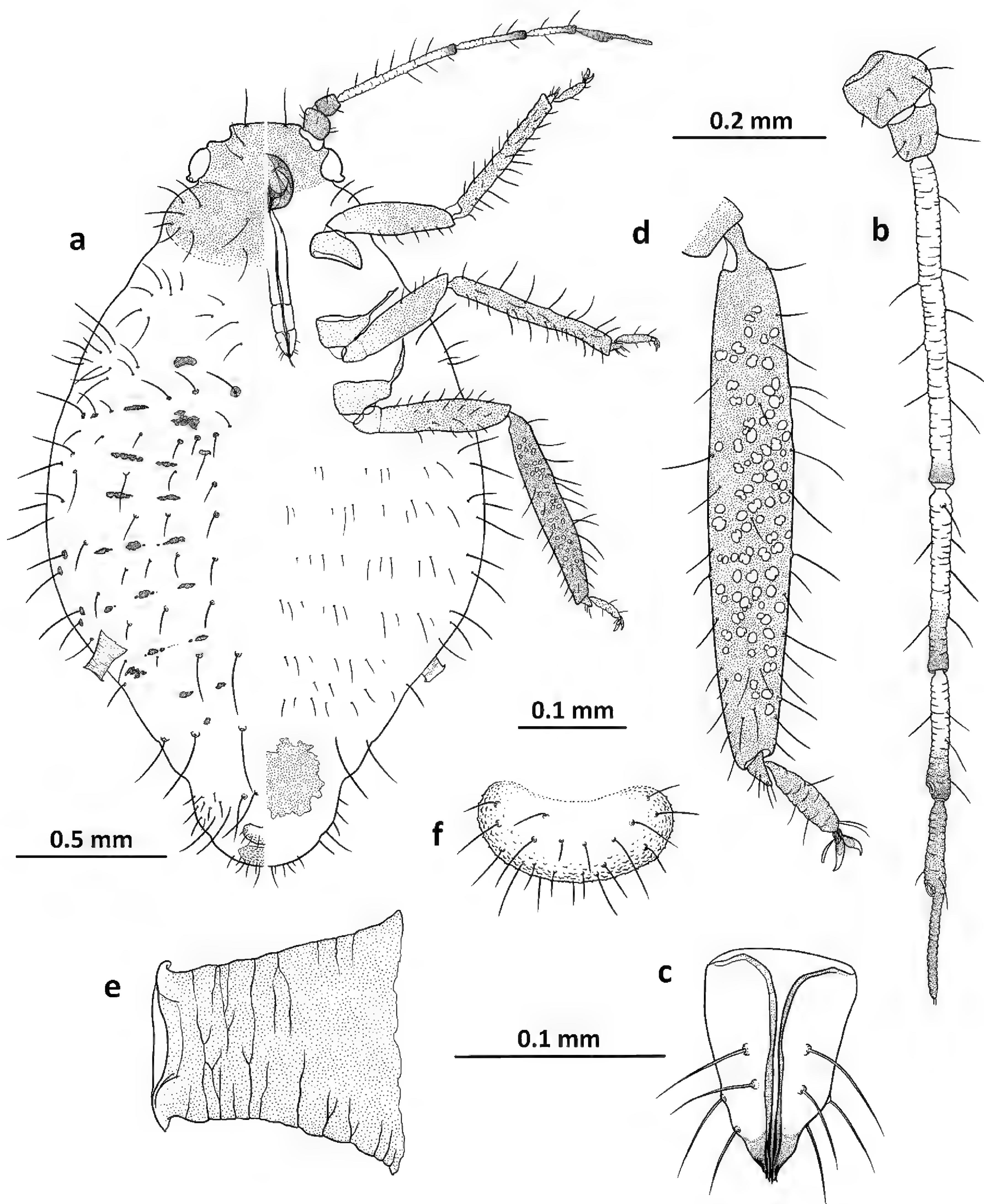
III–V covered by rounded secondary rhinaria: ANT III with 25–43 rhinaria, ANT IV with 13–23 rhinaria, ANT V with 13–18 rhinaria. Rostrum reaching middle coxae. ARS 0.11 mm long, 0.15–0.17 × ANT III and 0.64–0.68 × HT II, with 4 accessory setae (Fig. 2c). Legs with numerous fine and pointed setae 0.02–0.09 mm long. FEM-



**Fig. 2.** *Periphyllus brevispinosus* Gillette & Palmer, 1930, alate male. **a**. Head. **b**. Antenna. **c**. Apical segment of rostrum. **d**. Hind tarsus. **e**. Fore wing. **f**. Abdomen. **g**. Siphunculus. **h**. Cauda. **i**. Genitalia.

ORA III 0.73–0.77 mm long. TIBIA III 1.02–1.05 mm long. HT II 0.16–0.17 mm long. Distal part of tibiae with many short spinules; empodial setae spatulate; first tarsal chaetotaxy 5:5:5 (Fig. 2d). Fore wings with normal venation (Fig. 2e). Abdominal tergites membranous,

with large fused spinal sclerites, pleural sclerites very small, irregularly placed, marginal sclerites oval; sclerites on ABD VII–VIII fused in cross bars. Abdominal setae 0.06–0.18 mm long; marginal sclerites with 2–5 setae (0.09–0.18 mm long), pleural sclerites with 0–1



**Fig. 3.** *Periphyllus brevispinosus* Gillette & Palmer, 1930, oviparous female. **a.** General view. **b.** Antenna. **c.** Apical segment of rostrum. **d.** Hind tibia with pseudosensoria and tarsus. **e.** Siphunculus. **f.** Cauda.

setae (0.07–0.13 mm long), spinal sclerites with 2–3 (0.06–0.17 mm long) setae. Siphunculi 0.11–0.13 mm long and 0.11 width, truncate with developed flange, reticulated, except at base where reticulation transforms into flattened cells and coalescent with each marginal sclerite of ABD VI (Fig. 2g). Cauda 0.06–0.07 mm long, broadly rounded, with 14–15 setae 0.05–0.10 mm long (Fig. 2h). Genitalia well developed, strongly sclerotized with roundish, lobate parameres, covered by numerous spine-like setae. Basal part of phallus triangular, shortened, with numerous short spinules (Fig. 2i).

**Oviparous female** (Fig. 3). Colour in life: unknown; mounted specimens with head, legs, scleroites and siphunculi dusky. ANT dusky with apices of ANT III–V and ANT VI dark. Body 2.32–2.60 mm long and 1.40–1.55 mm width, pear-shaped (Fig. 3a). Head with 4 pairs of fine, pointed setae 0.06–0.19 mm long. ANT 6-segmented, 1.27–1.40 mm long (Fig. 3b), reaching ABD III–IV, 0.48–0.59 × BL. ANT IV 1.20–1.56 ANT V; ANT V always shorter than ANT VI; PT 0.92–1.16 × BASE; other antennal ratios: VI:III 0.54–0.67, V:III 0.35–0.50, IV:III 0.50–0.65. ANT I with 4–8 setae, ANT II with 3–5 setae, ANT III 0.40–0.48 mm long with 7–9 setae, ANT IV 0.24–0.28 mm long with 3–6 setae, ANT V 0.16–0.20 mm long with 3–4 setae, BASE 0.12–0.14 mm long with 2 setae, PT 0.13–0.15 mm long with 3 apical setae. ANT setae fine, pointed, 0.03–0.07 mm long. LS ANT III 1.25–2.33 × BD III. Rostrum almost reaching hind coxae. ARS 0.10–0.11 mm long, 0.20–0.25 × ANT III and 0.66–0.76 × HT II with 4 accessory setae (Fig. 3c). Legs with numerous fine and pointed setae, 0.03–0.10 mm long. FEMORA III 0.39–0.59 mm long. TIBIA III 0.70–0.80 mm long. HT II 0.13–0.16 mm long. Hind tibiae with 69–150 8-shaped pseudosensoria distributed on the whole length of tibiae. Distal part of tibiae with few short spinules; empodial setae spatulate; first tarsal chaetotaxy 5:5:5 (Fig. 3d). Abdominal tergites membranous. Abdominal setae 0.05–0.20 mm long; marginal sclerites with 2–5 setae (0.05–0.20 mm long), pleural and spinal sclerites with 3 setae (0.05–0.15 mm long). Siphunculi 0.11–0.12 mm long and 0.12–0.13 mm width, truncate, with weakly visible 2–3 rows of reticulations which at base transform into flattened cells and well-developed flange (Fig. 3e). Cauda 0.09–0.10 mm long, broadly rounded, with 20–25 pointed setae (Fig. 3f).

#### *Periphyllus negundinis* (Thomas, 1878)

Thomas, 1878: 10

Figs 4–5

*Periphyllus negundinis* is the most widely distributed in North America among Nearctic species of *Periphyllus* (Blackman & Eastop 2019); it has been recorded from Alabama, Arizona, California, Colorado, Connecticut, Delaware, District of Columbia, Florida, Illinois, Indiana,

Iowa, Kansas, Louisiana, Maine, Maryland, Michigan, Minnesota, Missouri, Mississippi, Montana, Nebraska, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oregon, Pennsylvania, South Carolina, South Dakota, Texas, Utah, Virginia, Washington, West Virginia, Wisconsin, and Wyoming (USA); Alberta, British Columbia, Manitoba, New Brunswick, Nova Scotia, Ontario, Prince Edward Island, Quebec and Saskatchewan (Canada) and Mexico (Mexico), being its main host plants *Acer negundo* (Essig & Abernathy 1952; Palmer 1952; Richards 1972; Smith & Parron 1978; Knowlton 1983); also collected from *A. pseudoplatanus* (Palmer 1952).

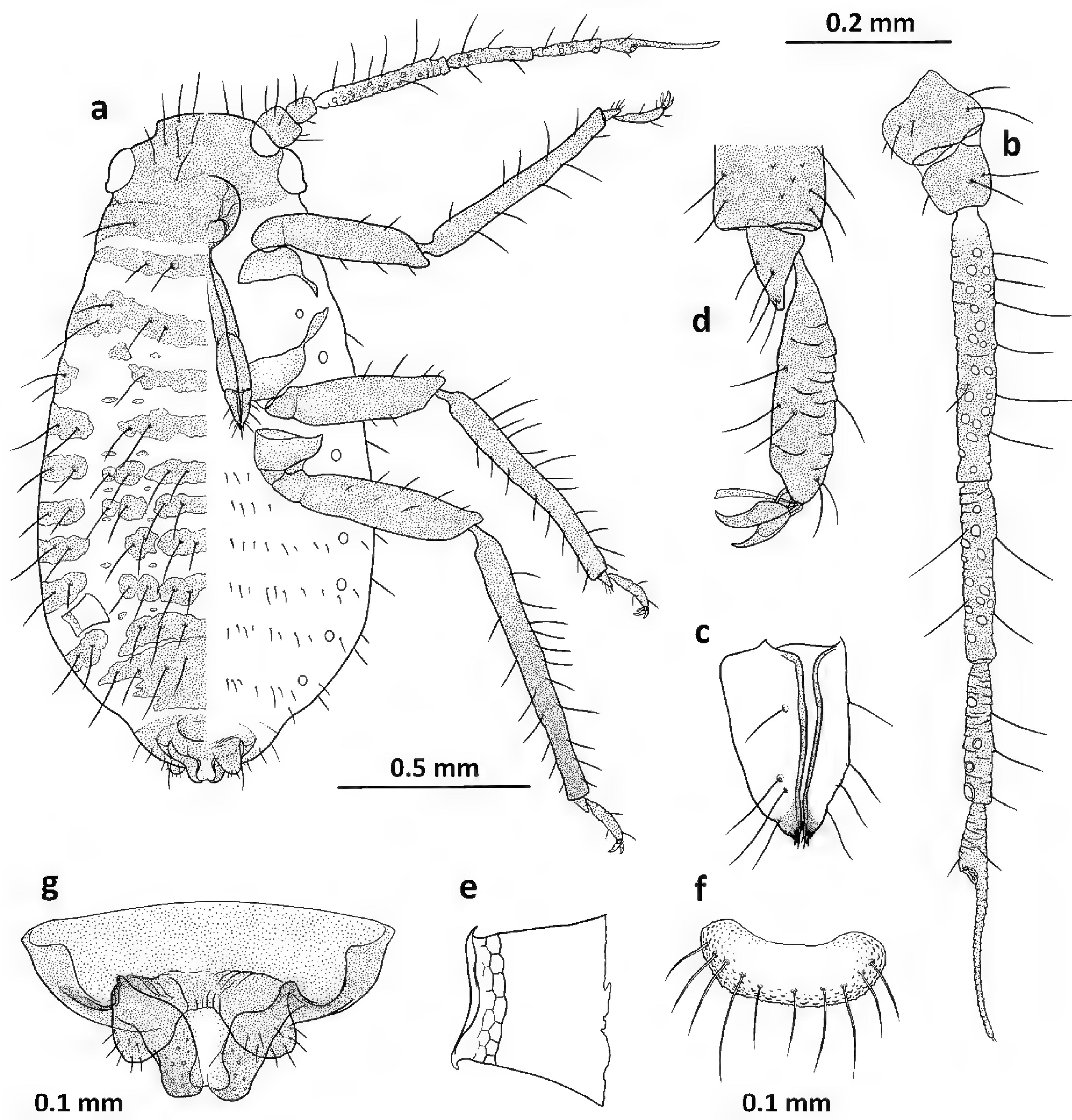
**Material examined.** CANADA, Manitoba, Winnipeg, 25 September 1963, on *Acer negundo*, A. G. Robinson leg., 1 oviparous female, BM 1964 630; 1 October 1964, on *Acer negundo*, A. G. Robinson leg., 2 apterous males, BM 1965 33.

**Apterous male** (Fig. 4). Colour in life: dark green (Essig & Abernathy 1952); mounted specimens with body pale with dusky sclerites; head, pronotum and genitalia dark. Antennae dark with basal part of ANT III paler. Legs dark with basal part of femora slightly paler. Body 1.60–1.72 mm long and 0.82–0.85 mm width. Head with 6–8 pairs of long fine, pointed setae 0.10–0.13 mm long (Fig. 4a). ANT 6-segmented, 1.20–1.27 mm long (Fig. 4b), reaching ABD IV, 0.70–0.79 × BL. ANT IV the same or slightly longer than ANT V; ANT V always shorter than ANT VI; PT 1.81–2.20 × BASE; other antennal ratios: VI:III 0.70–0.83, V:III 0.51–0.55, IV:III 0.54–0.72. ANT I with 4–5 setae, ANT II with 3–4 setae, ANT III 0.35–0.36 mm long with 7–8 setae, ANT IV 0.19–0.26 mm long with 3–4 setae, ANT V 0.18–0.20 mm long with 2–3 setae, BASE 0.09–0.11 mm long with 2 setae, PT 0.19–0.20 mm long with 3 apical setae. ANT setae fine, pointed, 0.02–0.13 mm long. LS ANT III 3.25–4.00 × BD III. The whole ANT III–V covered by rounded secondary rhinaria: ANT III with 16–23 rhinaria, ANT IV with 6–11 rhinaria, ANT V with 3–4 rhinaria. Rostrum reaching hind coxae. ARS 0.12–0.13 mm long, 0.34–0.36 × ANT III and 0.75–0.76 × HT II, with 4 accessory setae (Fig. 4c). Legs with numerous fine and pointed setae 0.06–0.16 mm long. FEMORA III 0.57–0.58 mm long. TIBIA III 0.77–0.81 mm long. HT II 0.16–0.17 mm long. Distal part of tibiae with few short spinules; empodial setae spatulate; first tarsal chaetotaxy 5:5:5 (Fig. 4d). Abdominal tergites membranous, with large, fused spinal sclerites, pleural sclerites smaller than spinal, irregularly placed, fused, marginal sclerites oval. Abdominal setae 0.03–0.21 mm long; marginal sclerites with 4–7 setae (0.03–0.21 mm long), pleural sclerites with 1–3 setae (0.08–0.15 mm long), spinal sclerites with 2–6 setae (0.07–0.14 mm long) (Fig. 4a). Siphunculi

0.17–0.18 mm long and 0.07–0.12 mm width, stump-shaped with 2 rows of reticulation and developed flange (Fig. 4e). Cauda 0.04–0.05 mm long, broadly rounded, with 10 setae (Fig. 4f). Genitalia well developed, strongly sclerotized with roundish, lobate parameres, covered by numerous spine-like setae. Basal part of phallus hook-shaped, shortened, with numerous short spinules (Fig. 4g).

**Oviparous female** (Fig. 5). Colour in life: mottled green, becoming darker as eggs mature within body (Essig &

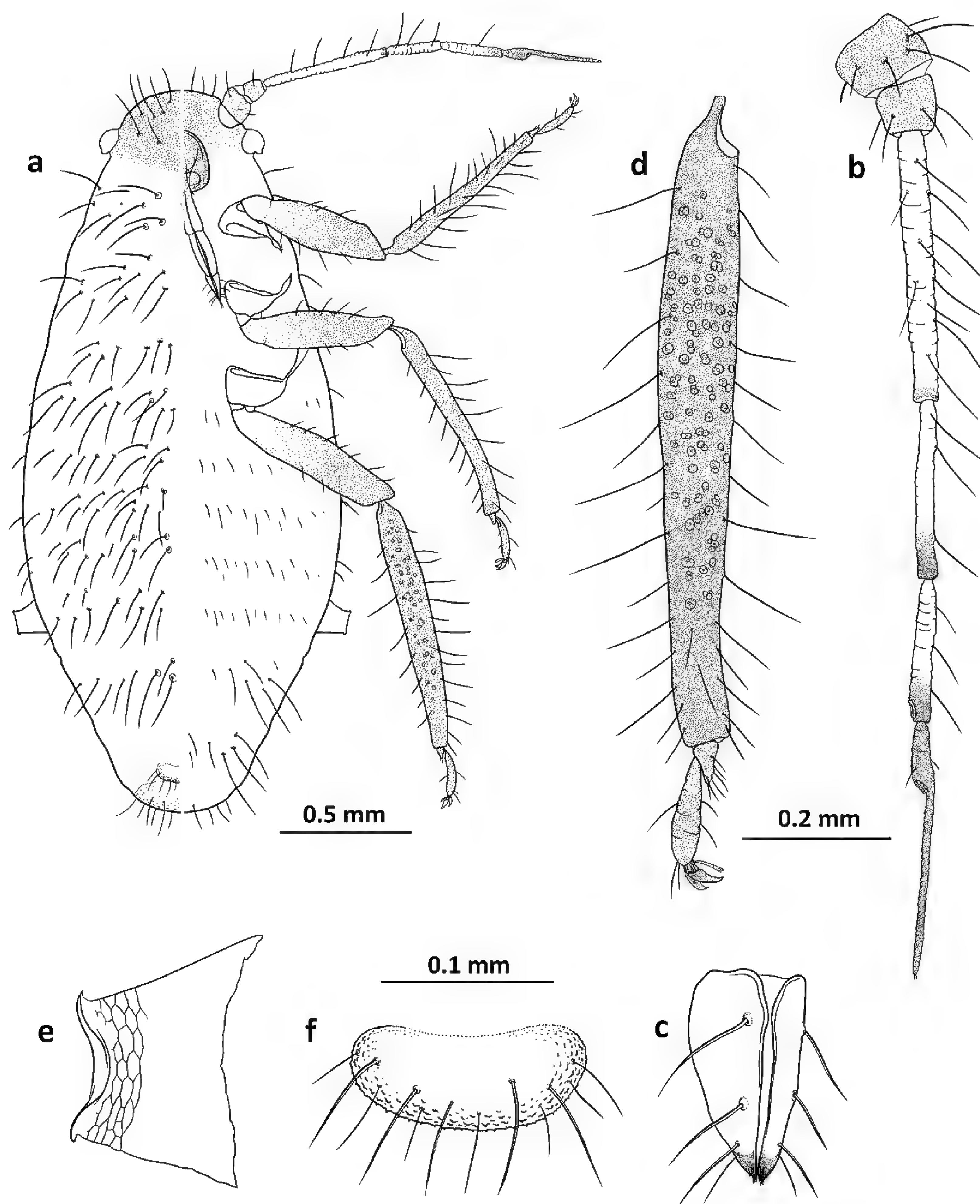
Abernathy 1952); mounted specimens with body pale with apices of ANT IV–V, ANT VI, tarsi and HTII dark. Body 2.55 mm long and 1.20 mm width, egg-shaped (Fig. 5a). Head with 5–7 pairs of fine, pointed setae 0.12–0.20 mm long. ANT 6-segmented 1.30–1.35 mm long (Fig. 5b), reaching ABD III–IV, 0.50–0.52 × BL. ANT IV slightly longer than ANT V; ANT V always shorter than ANT VI; PT 2.27 × BASE; other antennal ratios: VI:III 0.58–0.69, V:III 0.55–0.61, IV:III 0.58–0.69. ANT I with 6–8 setae, ANT II with 4–5 setae, ANT III 0.36 mm long with 8–10 setae, ANT IV 0.21–0.25 mm long with 3–4



**Fig. 4.** *Periphyllus negundinis* (Thomas, 1878), apterous male. **a.** General view. **b.** Antenna. **c.** Apical segment of rostrum. **d.** Hind tarsus. **e.** Siphunculus. **f.** Cauda. **g.** Genitalia.

setae, ANT V 0.20–0.22 mm long with 3–4 setae, BASE 0.11 mm long with 2 setae, PT 0.25 mm long with 3 apical setae. ANT setae fine, pointed, 0.02–0.12 mm long. LS ANT III  $3 \times$  BD III. Rostrum reaching middle coxae. ARS 0.13 mm long,  $0.36 \times$  ANT III and  $0.72–0.76 \times$  HT II with 4 accessory setae (Fig. 5c). FEMORA III 0.64 mm long. TIBIA III 0.89–0.90 mm long. HT II 0.17–0.18 mm long. Legs with numerous fine and pointed setae 0.04–0.15 mm long. Hind tibiae with 120–130 rounded pseudosensoria, sometimes fused and distributed on the whole

length of tibiae except basal and distal part. Distal part of tibiae with few short spinules; empodial setae spatulate; first tarsal chaetotaxy 5:5:5 (Fig. 5d). Abdominal tergites membranous. Abdominal setae numerous, fine, regularly placed all over abdomen, 0.06–0.22 mm long. Siphunculi 0.10 mm long and 0.15–0.16 mm width, stump-shaped, with 3–4 rows of reticulations and well-developed flange (Fig. 5e). Cauda 0.08 mm long broadly rounded, with 12 setae 0.05–0.15 mm long (Fig. 5f).



**Fig. 5.** *Periphyllus negundinis* (Thom., 1878), oviparous female. **a.** General view. **b.** Antenna. **c.** Apical segment of rostrum. **d.** Hind tibia with pseudosensoria and tarsus. **e.** Siphunculus. **f.** Cauda.

**Key to males of species of *Periphyllus* (native and introduced) known from the Nearctic**

1. Apterous ..... *P. negundinis*
- Alate ..... 2
2. Hind tibiae uniformly dark ..... 3
- Hind tibiae pale, dusky or dark only on base and apex ..... 4
3. Siphunculi reticulated on the whole length, PT 2.76–3.00 × BASE ..... *P. americanus*
- Siphunculi reticulated on apical 2/3, imbricated to wider base, PT > 3.0 × BASE ..... *P. californiensis*
4. Hind tibiae pale or dusky. Cauda helmet-shaped ..... *P. lyropictus*
- Hind tibiae dark on base and apex, pale at middle part. Cauda broadly-rounded ..... 5
5. PT 2.30–2.41 × BASE ..... *P. brevispinosus*
- PT 5.70–5.90 × BASE ..... *P. testudinaceus*

**Key to oviparous females of the species of *Periphyllus* (native and introduced) known from the Nearctic**

1. Cauda helmet-shaped ..... *P. lyropictus*
- Cauda broadly rounded ..... 2
2. PT 0.92–2.60 × BASE ..... 3
- PT 3.00–3.50 × BASE ..... *P. testudinaceus*
3. Body pear-shaped and dusky with dark ANT VI and apices of ANT III–V ..... *P. brevispinosus*
- Body egg-shaped and pale with dark tarsi, hind tibiae, ANT VI, and apices of ANT IV–V ..... 4
4. Siphunculi pale. On *Acer negundo* or *A. pseudoplatanus* ..... *P. negundinis*
- Siphunculi dark. On Asian ornamental maples ..... *P. californiensis*

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## Scientific note

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# New distribution range of the vulnerable wild goat (*Capra aegagrus* Erxleben, 1777) (Artiodactyla: Bovidae) to the south of its known extant in Iraq, with notes on its conservation

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**Abstract.** The wild goat (*Capra aegagrus* Erxleben, 1777) is a vulnerable ungulate confined to the rocky slopes of the Zagros Mountains forest steppes ecoregion in northern and northeastern Iraq (Kurdistan Region). Scattered populations had been reported from 31 sites distributed mainly in four Iraqi northern provinces; however, the species' current zoogeographical distribution and population trends are enigmatic. From August 2017 to April 2018, four new sightings of the wild goat were obtained from the foothills of the Zagros Mountains along the eastern and southeastern Iraq-Iran international borders. These new localities represent a new distribution range to the southernmost of the species' known extant in Iraq. Moreover, the newly discovered wild goat populations in eastern and southeastern Iraq almost certainly originated from the western Iranian populations assigned to the *Capra a. aegagrus* subspecies. Besides poaching, newly documented threats such as trapping and young capturing which severely affect the wild goat populations in Iraq are discussed.

**Key words.** Bovidae, *Capra aegagrus*, protected areas, ungulates, wild mammals of Iraq.

## INTRODUCTION

The wild goat (*Capra aegagrus* Erxleben, 1777) is a threatened ungulate restricted to the mountainous habitats of central Afghanistan, southern Pakistan, west through Iran, western Turkmenistan, northern Iraq, the Caucasus region (Armenia, Azerbaijan, northeastern Georgia, and southern Russia), and southwestern Turkey (Weinberg et al. 2008; Macar & Gurkan 2009). In Arabia, the species once occurred in Lebanon and Syria, United Arab Emirates, and Jordan, but is now extinct in these regions (Harrison & Bates 1991; Grubb 2005).

In Iraq, the species inhabits the rocky slopes, mountain gorges, wooded hills, coniferous and Mediterranean shrubland of the Zagros Mountains mainly in the extreme northern and northeastern Iraq (Kurdistan Region) (Al-Sheikhly et al. 2015). Previously, it has been reported from Shanidar caves, mountain slopes near Zawitha, Sarsank and Amadiya in Dohuk province, Baradust Mountain, Barzan area, Zagarta Mountain, Shaglawa, Harir Dagh, Rawanduz, Safin Dagh and Bekma Dam in

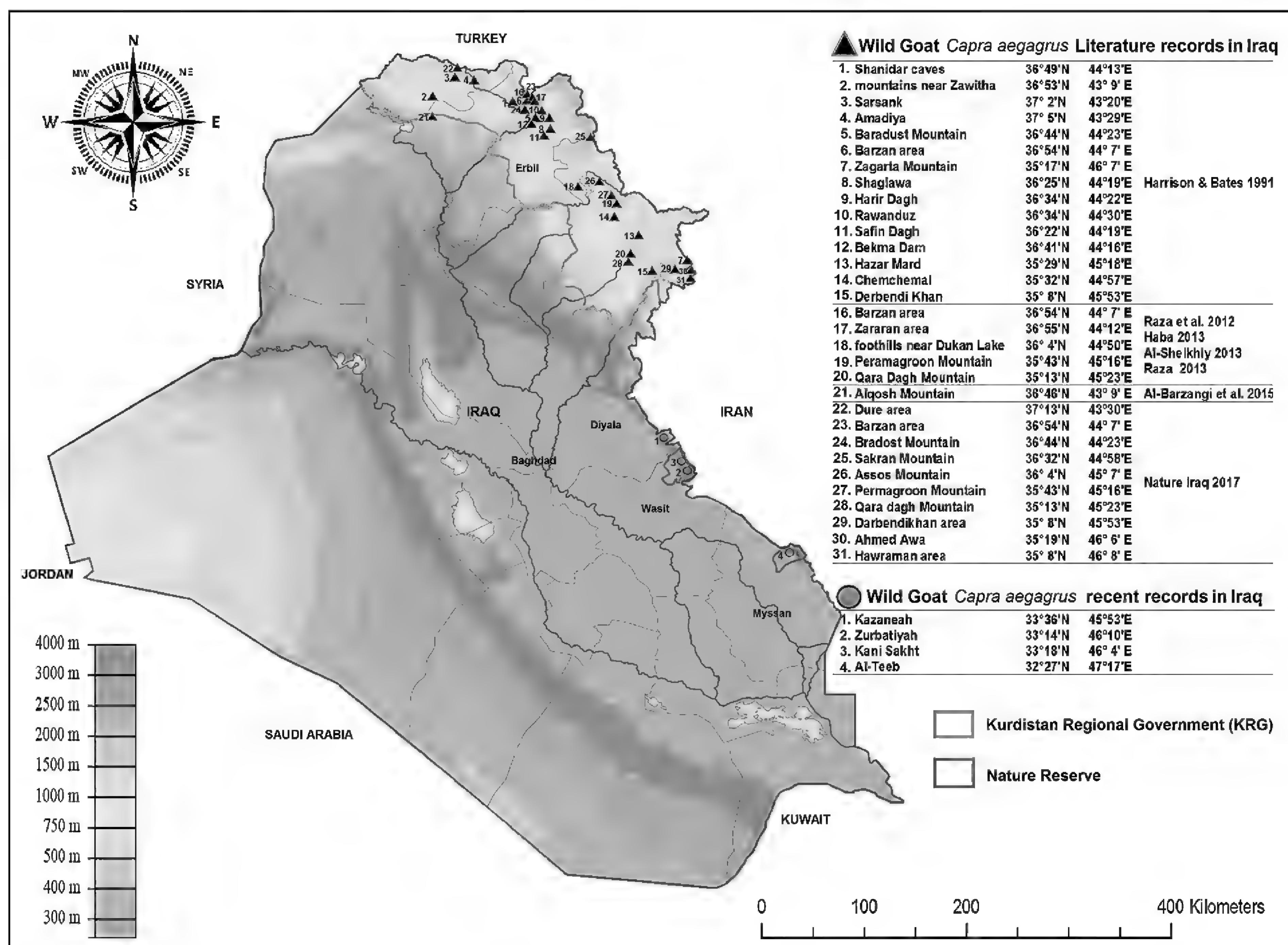
Erbil province, recorded also from Hazar Mard, Chemchemal and Derbendi Khan in Sulaimaniyah province (Harrison & Bates 1991). Scattered small populations have been reported from Barzan area, Zararan (Zerara), foothills near Dukan Lake, Peramagroon, and Qara Dagh mountains during 2010–2012 (Al-Sheikhly 2012b; Raza et al. 2012; Haba 2013; Raza 2013). More recently, a small population of wild goat has been found in Buzan Valley in the Alqosh Mountain, a newly discovered locality for this species in Nineveh province in northern Iraq (Al-Barzangi et al. 2015). Furthermore, wild goats have been reported for ten Key Biodiversity Areas (KBAs) in the mountain chains of the Kurdistan Region in northern Iraq (Nature Iraq 2017) (Fig. 1). The wild goat is listed as Vulnerable (VU) by the International Union for Conservation of Nature (IUCN) Red List due to rapid population decline attributed to over-exploitation (mainly by poaching), competition for gazing areas with domestic livestock, disturbance, and habitat destruction (Weinberg et al. 2008).

## New sites for wild goat in eastern and southeastern Iraq

Four new sites for wild goat have been recently identified within the Zagros Mountains forest steppes ecoregion along Iraq-Iran eastern and southeastern international borders, the foothills of (i) Zurbatiyah (Zurbatia), (ii) Kani Sakht, (iii) Kazaneah, and (iv) Al-Teeb (red-circled sites in Fig. 1).

The foothills of Zurbatiyah (site 2 in Fig. 1) and Kani Sakht (site 3 in Fig. 1) are extending to the northeast of Badrah district in Wasit province, Kazaneah (site 1 in Fig. 1) extends to the southeast of Mandli district in Diyala province (ca. 140 km far from Baghdad), and Al-Teeb (site 4 in Fig. 1) extends to the to the north of Myssan province. The general landscape of sites 1–3 is characterized by rocky slopes, rocky outcrops and vegetated hills, while site 4 is mainly dominated by broad rocky and gypsum valleys, arid plains, and grasslands. These habitats seem to provide food resources, water streams for drinking, hiding places, grazing areas, and possibly mating sites for wild goats.

Our intensive interviews with local hunters and villagers indicated that several scattered herds of 15–80 wild goats are frequently chased and persecuted by local hunters along the foothills of eastern and southeastern Iraq where small resident populations may be present. In August 2017, two adult males and an adult female with twin offspring (ca. 3 weeks old) were shot in the rocky slopes of Zurbatiyah foothills by local hunters (Fig. 2b). In February 2018, three adult males were shot in the foothills of Kani Sakht (Fig. 2e–f), and four adult males were shot in the foothills of Kazaneah in November 2018 (Fig. 2g). Recent reliable reports indicate that scattered nomadic herds of wild goats were frequently observed and chased by local hunters in the foothills of Al-Teeb in March–April 2018 which represents the southernmost sighting of this species in Iraq (BM Al-Taei, pers. comm. 2019). All of the examined wild goat male carcasses showed a russet-brown pelage with black stripes on the back, around the withers, on the front of legs and on the edge of the pale-brown belly, and black foreheads with long black beards and long horns curved backwards.



**Fig. 1.** Literature and recent records of Wild goat *Capra aegagrus* Erxleben, 1777 in Iraq.

The current zoogeographical range of wild goat in Iraq is represented by a patchy distribution pattern of scattered populations of the subspecies *C. a. aegagrus* which mainly exist in northern and northeastern Iraq (Kurdistan Region) (Al-Sheikhly & Haba 2014; Al-Sheikhly et al. 2015). However, the Iraqi largest sedentary populations of wild goat are known from Peramagroon, Qara Dagh, and Barzan mountains where they are protected by the Kurdistan Forestry Police and preserved by tribal communities for decades in the latter mountain (Raza et al. 2012; Haba 2013). The Forestry Police of Mergasur estimated more than 1000 wild goats thriving in Barzan area, from which over 200 individuals had died due to the outbreak of goat plague or Peste des Petits Ruminants (PPR) in August 2010 and in 2011 (Nature Iraq 2017).

During a field expedition on 26<sup>th</sup> of August 2017, a herd of 94 wild goats (46 adult males; 31 adult females; 17 juveniles) was counted using point count method (e.g., Gundogdu 2011) in an area of 400 ha (0.235 individual/ha) of wooded mountain slopes in Zerara (Barzan area) in Erbil province in northern Iraq (Fig. 2c–d). Adult males ( $\geq 2$ –3 years) were representing ca. 50% of the observed population (Fig. 2a); a ratio supported by interviews with Kurdish villagers and forestry policemen. In spite of surveying only a small proportion of Zerara area, our count was higher than the previously known estimate (80 individuals/summer 2010, Nature Iraq 2017). The increase of wild goat population size in Barzan area may be attributed to the protection provided by the local communities and Kurdistan Regional Government (KRG); yet, further monitoring may reveal better estimates. However, our newly discovered wild goat localities in eastern and southeastern Iraq are distant ca. 200 km away from the largest resident populations in northern Kurdistan and represent the southernmost distribution range of the species in Iraq.

The newly discovered wild goat populations in eastern and southeastern Iraq most certainly originated from Iranian populations that inhabit the Zagros Mountains of western Iran. Wild goat is widely distributed in Iran; it has been recorded from 31 provinces and reported from 190 protected areas throughout the country (Karami et al. 2008; Yusefi et al. 2019). In Ilam province in western Iran, the Kolan (Golan) Protected Area (33°23' N, 46°9' E) and Bina and Bijar No-hunting area (33°41' N, 45°56' E) are the most adjacent protected areas to our sites 1–3, while Dinar Kuh (Dinar Kooh) (32°50' N, 47°20' E) Protected Area is the closest protected area to our site 4 (Darvishsefat 2006; Yusefi et al. 2019; UNEP-WCMC 2020) (Fig. 1). However, Dehloran city is placed between Dinar Kuh Protected Area and site 4; therefore, wild goats are probably using the free area of ca. 7 km of Iraq-Iran international border to reach site 4. Furthermore, the free area of the international border seems important for wild goat to move from well protected areas and no-hunting zones in Iran to Iraq.

In Iran, the mating season of wild goat starts in mid-fall (October–December) and in November in southeastern Turkey, when both sexes aggregate in herds and males' courtship display starts (Korshunov 1994; Ziae 2008; Esfandabad et al. 2010). The dominant large adult males push the young and sick goats out of the herd which migrate to adjacent habitats in search for new hiding and grazing places. Furthermore, after mating, the large adult males move to new areas to spend the summer (Gundogdu 2011). The increased conspecific competition among wild goat males may force some individuals from the Iranian western populations to migrate to eastern and southeastern Iraq. Moreover, as we mentioned before, the high numbers of different-aged wild goat males that were recently hunted in eastern and southeastern Iraq seems to support the claim that migrated animals survived the severe poaching and were able to establish new populations in eastern and southeastern Iraq. In addition, wild goat females with recently born young are frequently chased and trapped by local poachers, which confirms the breeding of this species within the territory of Iraq. Therefore, urgent and increased conservation and protection actions should be taken to save these new populations.

Esfandabad et al. (2010) suggested altitudinal and seasonal migration behaviors of wild goat as an adaptation to survive in extreme temperature and to make use of available resources throughout the year. In winter (January–March), the low accessibility to food resources due to a thick layer of snow at higher elevations forces the animals to use lower altitudes where they become exposed to natural predators and poachers (Esfandabad et al. 2010).

It is worth noting that the wild goat population of western Iran was assigned to the nominotypical subspecies *C. a. aegagrus* Erxleben, 1777, while the eastern and southeastern ones were assigned to *C. a. blythi* (Hume, 1875) (Ellerman & Morrison-Scott 1951; Heptner et al. 1988). Grubb (2005) synonymized both subspecies, and therefore, only one subspecies (*C. a. aegagrus*) occurs in Iran (Yusefi et al. 2019). Furthermore, the domestication process of goats was intensively studied by Naderi et al. using mtDNA (2007; 2008). The wild goat populations of eastern and southeastern Iraq is therefore assigned to the Iranian subspecies *C. a. aegagrus*.

## Threats on the wild goat population in Iraq

### Poaching

Hunting of wild goat has been identified as a major threat on the species survival (Weinberg et al. 2008; Gundogdu 2011). The illegal hunting (shooting, trapping, and catching by dogs) is a major threat on the species in Iran which increases during the rutting season in fall, when trophy males are easier to detect by poachers (Ziae 2008). The wild goat population in Iraq had remarkably decreased

due to excessive poaching compared to the 1920s (Hatt 1959; Al-Sheikhly 2012c). In northern Iraq (Kurdistan Region), most of the wild goat populations were affected by habitat destruction and disturbance that occurred during Iraq-Iran conflict in the 1980s (Al-Sheikhly et al. 2015). However, the hunting of wild goats was prohibi-

ed by the Kurdistan Regional Government in the 1990s (Al-Barzangi et al. 2015). In eastern and southeastern Iraq, the hunting of wild goats and other sympatric bovid species is continued and seems to be uncontrolled by the local authorities and warrants urgent governmental protection. Two of our sites, Zurbatiyah and Al-Teeb



**Fig. 2.** **a.** Adult males wild goat *Capra aegagrus* Erxleben, 1777 grazing on rocky slopes of Zerara area in Erbil province in northern Iraq. **b.** An adult male shot in Zurbatiyah foothills (red-circled site 2 in Fig. 1). **c-d.** Females with kids observed in Zerara area in Erbil province in northern Iraq. **e-f.** Adult males shot in the foothills of Kani Sakht (site 3 in Fig. 1). **g.** An adult male shot in the foothills of Kazaneah (site 1 in Fig. 1). **h.** Asiatic Mouflon *Ovis orientalis gmelini* shot in the foothills of eastern Iraq. **i.** A wild goat kid captured from the foothills of eastern Iraq in order to be raised as a pet. Photos: a-d: ©Omar Al-Sheikhly; b-i: ©Basheer Mohammad Al-Taei.

(Zubaidaat and Teeb Oasis) have national importance for wildlife conservation. Both sites have been designated as Key Biodiversity Areas (KBAs no. 57 and 67) respectively and declared as nature reserves by the Ministry of Environment of Iraq (Nature Iraq 2017; UNEP-WCMC 2020).

Besides wild goat, the Vulnerable Asiatic mouflon *Ovis orientalis gmelini* (Blyth, 1841) (Fig. 2h) and the Vulnerable Persian goitered gazelle *Gazella subgutturosa* (Guldenstaedt, 1780) are targeted by local hunters where and whenever possible in order to be consumed as food or raised as pets (Al-Sheikhly 2012a; c). Furthermore, the Vulnerable Arabian Sand Gazelle *G. marica* (Thomas, 1897) probably exists in eastern and southeastern Iraq (Fadakar et al. 2019). It is worth mentioning, that hunting the aforementioned species is banned by the Iraqi Wild Animals Protection Law (no. 17 issued on 15<sup>th</sup> of February 2010), but the weak enforcement encourages hunters to pursue their illegal quest. The local hunters who are equipped with modern hunting rifles and fleets of all terrains are extensively searching for wild bovids in the foothills and steppes of eastern and southeastern Iraq throughout the year, with extensive poaching occurring mainly in winter and spring (January–June). Poaching of wild goat in eastern and southeastern Iraq is extensively practiced in winter (seven males/November–February 2018) when wild goats seem to abandon the higher grounds of the adjacent protected areas in western Iran to retreat to lower altitudes in eastern and southeastern Iraq.

### Trapping

In our recent investigation we found that trapping has emerged as a newly documented threat on wild bovid populations in Iraq. The solitary, nomadic, and rutting wild goats, Asiatic mouflons, and Persian goitered gazelles are ambushed and trapped by large nets fired from net-modified rifles or taken by a long chase via motorcycles. The trapped animals are sold in the local animal markets or exported to the neighboring Arabian countries as pets. Our interviews with the local animal traders revealed that the prices of the trapped animals are varying based on sex and age. The price ranges from \$300–500 for wild goat juveniles and adult female Persian goitered gazelles, to \$800–900 for adult female wild goats, and \$400–500 for adult male wild goats.

### Capturing/collecting of young from the wild

Our interviews, personal communications and correspondences with local hunters indicated that parturition takes place in early April to mid July in the wild goat populations of eastern and southeastern Iraq which seems concurrent to those of western Turkmenistan (Korshunov 1994) and southeastern Turkey (Gundogdu 2011). At that time, foot patrols of local trappers/collectors are search-

ing for wild goat pregnant females and/or their newly born not-weaned offspring in the rocky caverns and foothill cliffs of eastern Iraq. The interviews indicate that the kidnapped wild goat juveniles are kept to be raised as pets or sold in the local animal markets where many may die due to irresponsible care (Fig. 2i). It is worth mentioning that the capturing of the ungulate young by local trappers seems to be a common illegal practice in western and central Iraq which requires further actions (Al-Sheikhly 2012a).

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## Scientific note

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# A note on the wild canids (Carnivora: Canidae) of the Horn of Africa, with the first evidence of a new – forgotten – species for Ethiopia *Canis mengesi* Noack, 1897

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**Abstract.** The first ever reported observation of a member of the genus *Canis* well in the interior of the Danakil area (Ethiopia) offers the opportunity to revise available evidence about the existence of a neglected species of small-sized ‘jackal’ in the Horn of Africa. A review of historical zoological literature led to assign this small-sized, reddish jackal to *Canis mengesi* Noack, 1897, originally described from inner Somaliland. Geological and geomorphological considerations support the distinctiveness of the Red Sea coastal jackal *Canis anthus riparius* Hemprich & Ehrenberg, 1832, typical of the narrow alluvial, sandy coast, while *Canis mengesi* is found in the volcanic rocky habitat prevailing over most northern Afar (Danakil, Ethiopia).

**Key words.** *Canis mengesi*, *Canis riparius*, Danakil, geomorphology, taxonomy.

Knowledge of mammals in some parts of the Horn of Africa is still very sparse, and gaps exist even with respect to species diversity among the most visible mammals. Regarding canids, one of the best studied mammal group worldwide, a short note on an unusual wolf-like individual observed in coastal Danakil Eritrea (Tiwari & Sillero Zubiri 2004) should have promoted more interest regarding the issue of alpha diversity among canids in the Horn of Africa, but nothing apparently was done and this observation remained unidentified. Currently, much scientific interest concerning the intriguing African ‘golden jackal’ group, has been attracted by new phylogenetic data that allied this canid closer to the Eurasian wolf *Canis lupus* rather than the Eurasian golden jackal *Canis aureus* (Viranta et al. 2017).

The object of this note is another observation of a single individual of an unusual small-sized canid in the interior of northern Afar (Danakil) region of Ethiopia, done by one of us (L.L.) on January 1<sup>st</sup> 2018, along the road between the Massif of Dadar and the Massif of Masca (about 12°43' N, 41°08' E). A few photos allow us to discuss some details concerning this individual while attempting a taxonomic identification. This small canid was observed along a recently asphalted road about 150 km

south of Lake Afrera, an area lacking data concerning the presence of any member of the genus *Canis* (cfr. Yalden et al. 1980). The small size of this individual canid is evident from comparison with the horizontal traffic signals (Fig. 1).

The general reddish coloration may suggest a red fox *Vulpes vulpes* – a species that is probably part of the Eritrean fauna (Gippoliti 2020a) yet unlikely to inhabit the Danakil Region – but the blackish end of the tail, the lack of black behind the ear, the body building and body proportion (Fig. 2) led to reject this hypothesis.

As evidenced by photos, the canid was observed in rocky habitat typical of the central and northern rocky plateau of Afar. The Danakil depression is floored principally by Pliocene volcanic rocks of the *Afar Stratoid series* (Barberi & Santacroce 1980). The Afar Depression, between the Red Sea and the Ethiopian plateau, is covered by Tertiary and Quaternary volcanic rocks. The Danakil Alps run parallel to the coast, forming a strong barrier between the interior area and the sandy and alluvial coastal region (Fig. 3) (Lupi 2009, 2012).

Unlike Tiwari & Sillero-Zubiri (2004), we examined the classical zoological and taxonomic literature, a valuable source of data concerning morphological diversity



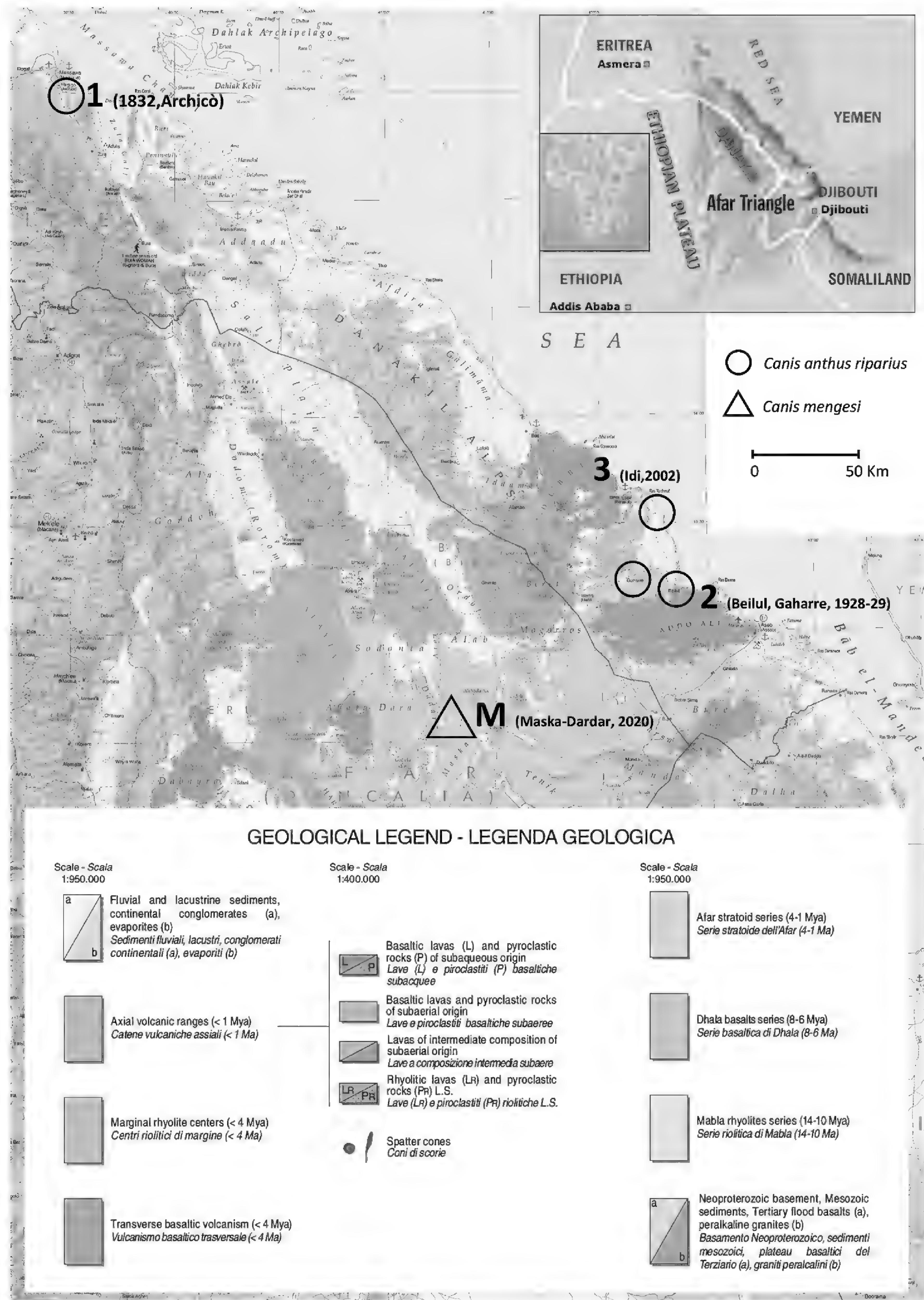
**Figs 1 (left) and 2 (right).** Two photos of the same individual canid. Note the small size and habitat characteristics where this canid individual was observed. Photos: Luca Lupi.

that is often overlooked by recent research (Gippoliti 2020b). We found that a canid taxon with the characters shown in the photo was described from the Horn of Africa at the end of the 19th century. In 1897, the German zoologist Theodor Noack described two new canid species from several living specimens captured in Somaliland by Joseph Menges, a collaborator of the famous Carl Hagenbeck (Noack 1897). The larger species was named *Canis hagenbecki* while the smaller one became *Canis mengesi*. These descriptions were based on numerous living specimens and several skulls in the possession of Noack. According to Noack (1897), *Canis mengesi* is smaller and shorter-legged than *C. hagenbecki*. His diagnostic characters, especially compared with *C. hagenbecki*, include the following: snout is shorter and the longer ear has a slenderer tip. The hind quarters are kept low, so that the gait of the animal resembles a hyena. Hair on the back is short, with a dark spot on the middle tail, but without or at least with only a minor black tip of the tail. Body colour is reddish yellow or reddish grey, including the nasal area and the rear of the ears. Forehead red grey, hair on the back paler with more yellow hair tips, the brown of the hair tips hardly visible if present at all. Lower lip is brown, upper lip white, iris of the eye yellowish red with a grey hue. Legs are yellowish red, not darker on the forelegs, hardly paler on the inner sides. A dark collar on the neck is absent. Breast and belly are paler than the flanks. The species digs cavities in the soil. The vocalization is similar to *C. hagenbecki*, but more whining, rather like a young dog. Skull is slender, with an only weakly curved profile, nasal bones somewhat longer than maxillae, and broader in front than in the middle. Occiput as in *C. hagenbecki* and *C. anthus*. Pterygoid process more widely spaced from each other than in *C. hagenbecki*. Upper premolars 2 and 3 with additional cusp, as lower premolar 4 that has 2 additional cusps. Noack failed

to provide details on types, and the exact provenance of *Canis mengesi* – as those of *C. hagenbecki* – remained vague – Somaliland, Küstengegenden, Inneres? (Noack, 1897: 518). Heck (1899) under the name of *Canis hadramaticus* Noack published a photo – perhaps the only one so far published – of one of the captive *Canis mengesi* studied by Noack, showing a relatively massive head compared to body and legs, and the white area over the lips (Fig. 3).

Some years later Hilzheimer (1906) not only accepted *C. mengesi*, but on the basis of the study of two more skulls (ex-captive animals captured in Somaliland) in the museum of Stuttgart, he described a new subspecies, *Canis mengesi lamperti*. With 125 and 121 mm their basilar lengths are even smaller than the type of *Canis mengesi*, which they resemble except that their nasalia do not extend as far back as the maxillary (Hilzheimer 1906). According to Hilzheimer (1906), *Canis mengesi* is the smallest true jackal, which forms a group of its own within the golden jackal group due to its color and skull shape. Its native range is unknown but could be the interior of northern Somalia. Maximum basilar length of its skull is 132 mm. He described *C. mengesi lamperti* as very small, as red as a fox, black in midline of back, very long ears, no black markings and tail with dark brown tip (Hilzheimer 1908).

The mounted skin of the holotype of *Canis mengesi lamperti* (SMNS 2394) is shown here for the first time as far as we know (Fig. 5), while its skull was shown by Hilzheimer (1908: table II). It is a male from Joseph Menges' expedition to Somaliland that lived in a zoo in Stuttgart and was donated by Nill to the museum in 1897 (Dieterlen et al. 2013). The meagre evidence so far available does not allow a clear assessment of the minor morphological differences between the two described taxa of *mengesi* and our own observation, yet we can conserva-



**Fig. 3.** Geomorphological map of Danakil (Lupi 2012), showing our visual record of *Canis mengesi* (triangle), and those assigned to *Canis anthus riparius* (circle).



**Fig. 4.** A living *Canis mengesi* at Berlin Zoo before his formal description by Noack. From Heck (1899) who labeled it *Canis hadramauticus* Noack. Note the white area over the lips, which was emphasized by Noack (1897) in his description of *mengesi*.

tively propose that a small reddish member of the genus *Canis* occurs in Danakil, Ethiopia and Somaliland and his name is *Canis mengesi* Noack, 1897.

The taxonomic history of *Canis mengesi* is quite typical of the systematic attitude of the previous century. Anderson (1902) studied a skin and skull of *C. mengesi* (not one of the typical series, apparently) in Berlin and maintained it as specifically distinct, a position that also De Winton (footnote in Anderson 1902: 220) clearly accepted, changing his previous opinion “In my paper on African Canidae, *C. mengesi* was doubtfully referred to this species [*C. variegatus*]. Further material has enabled me to see my mistake, and I fully agree with the view here expressed”. Anderson (1902) provided the following measurements taken on the mounted skin: head and body length 510 mm, tail length 223 mm, height at shoulder 290 mm, ear length 75 mm anterior and 90 mm posterior. Interestingly, some of these measurements are lower than those reported for the red fox *Vulpes vulpes* in Egypt (Osborn & Helmy 1980). The skin (no number but skull n. 6073) was described by Anderson (1902: 219–220) as follow “Greyish yellow throughout on the trunk, but many of the hairs with long black tips; slightly rufous on the upper surface of the muzzle and white along the upper lip and on the side of the face before the eyes; chin and throat white, but a brown area on the middle of the upper lip. Side of head below the ears yellow, back of ears yel-

low with black hairs intermixed. Inside of ears clothed with white hairs. A tendency to form a dark collar. Fore limbs bright yellowish, but with a faintly dark area down the front to near the wrist. Outsides of hind limbs yellow. Under parts white, with the exception of the base of the throat in front. Tail concolorous with the body, towards the tip broadly marked with dark blackish brown; the black spot on its dorsal surface well defined.” Schwarz (1926) synonymized several taxa, among them *mengesi*, under the name *Canis aureus riparius* Hemphrich & Ehrenberg, 1832. Glover Allen (Allen 1939), followed Schwarz and maintained *mengesi* as a synonym of *Thos aureus riparius* but considered *Thos lamperti* distinct at the specific level although stressing the need for further studies that never happened. Therefore Coetzee (1971) felt justified to state that “*Canis mengesi lamperti* [...] is regarded here as a synonym of *C. a. riparius*”, apparently without justification, but *de facto* relegating the taxon to oblivion.

After reviewing the few available data and the photographic evidence here presented, it seems reasonable to conclude that

1. A small-sized member of the genus *Canis* is found in the interior of Ethiopian Danakil,
2. Its reddish color, size and pattern generally agree with that of a forgotten taxon, *Canis mengesi*, so far historically known only from the interior of Somaliland,
3. Photographic evidence offered by Tiwari & Siller-Zubiri (2004) seems to confirm that *Canis mengesi* is a distinct taxon from the one occupying the coastal plain zone of Eritrea (i.e., *Canis anthus riparius*),
4. This new record may indicate that *C. mengesi* is a specialist of arid rocky habitats.

Apparently for the first time in decades we propose a much richer diversity of African canids taxa than usually recognized, particularly of the genus *Canis* – now that the species *mesomelas* and *adusta* are separated in the genus *Lupulella* (Atickem et al. 2017; but see also Machado & Teta 2020). The few data concerning golden



**Fig. 5.** Holotype of *Canis mengesi lamperti*, courtesy C. Leidenroth, State Museum of Natural History Stuttgart

jackals in the Eritrean coastal region (Fig. 3), historically assigned to the taxon *riparius*, are clearly limited to the sandy alluvial coastal plain while we documented a totally different canid in interior Danakil, that has been never observed in the much better studied coastal region. We propose therefore that the so far under-appreciated geomorphological diversity of the Horn of Africa is one of the reasons to explain the presence of several lineages of *Canis* (Gippoliti 2018). In agreement with Groves et al. (2017) and Gippoliti (2019), we think that such diversity is better described by ranking these lineages as species, and this is particularly the case with *Canis mengesi* whose dwarf size, specialized habitat and hair color is well outside the known variability shown by the 'African golden jackal/wolf' *Canis anthus* Cuvier, 1820 as it is now universally understood.

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### Scientific note

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## Further records of a poorly-known insular endemic skink *Lipinia macrotympanum* (Stoliczka, 1873) (Squamata: Scincidae)

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**Abstract.** The little-known, insular endemic skink *Lipinia macrotympanum* (Stoliczka, 1873) was recorded recently from two new localities in Great Nicobar Biosphere Reserve (GNBR) further south of the previously known localities. Based on these observations, new data on morphology, natural history and distribution are presented and it is suggested to be considered as an endangered species based on the IUCN assessment criteria.

**Key words.** *Lipinia macrotympanum*, new locality records, distribution map, Nicobar archipelago.

The skink genus *Lipinia* Gray, 1845 is represented by 32 species which are distributed from the Nicobar Islands in the West to Papua New Guinea in the east (Uetz et al. 2019). Of these, the westernmost representative of the genus, *Lipinia macrotympanum* was described originally by Stoliczka (1873) as *Mocoa macrotympanum* from “South Andaman, on a sandy beach in Macpherson’s Straits”. Later, Biswas & Sanyal (1977) reported a specimen of this species erroneously identified as “*Lygosoma quadrivittatum*” (now *Lipinia quadrivittata*) from Campbell Bay, Great Nicobar. Subsequently, Das (1997) reported the rediscovery of *Lipinia macrotympanum* based on his new collection of a specimen from Pulo Ulon, Little Nicobar and the specimen reported by Biswas & Sanyal (1977). Ever since this report, *Lipinia macrotympanum* was never recorded from the Andaman and Nicobar Islands. Herein, we report on two specimens of *Lipinia macrotympanum* based on our field observations and collected specimens. One of the individuals reported here (ZSI/ANRC/T/3709) was illustrated by Rangasamy et al. (2019) in their list of herpetofauna of the Andaman and Nicobar Islands.

Faunal surveys spanning 10–15 days were conducted on islands of the Nicobar archipelago intermittently between 2015 and 2018. Two of three specimens of *L. macrotympanum* recorded during these surveys were collected, preserved and deposited in the holdings of the Zoological Survey of India, Andaman and Nicobar Regional Centre (ZSI ANRC). The collected specimens were measured with a vernier caliper and a Leica stereomicroscope to the nearest 0.1 mm. The following characters were recorded: Snout tip to vent (SVL), trunk length from axilla to groin (AG), tail length from vent to tail tip (TL), head length (HL), head width at jaw angle (HW), head depth (HD), eye diameter (ED), tympanum diameter (TYD), eye-nostril distance (EN), eye-snout distance (ES), eye-tympanum distance (ETY), forelimb length (FLL), hindlimb length (HLL), inclusive of femur length (from groin to knee) (FEL) and tibia length (from knee to heel) (TBL), finger lengths (F1–F5), toe lengths (T1–T5), midbody scale-rows (MSR), nuchals (NU), supralabials (SL), infralabials (IL), ventrals (V), supraoculars (SO), prefrontals (PRF), subdigital lamellae under toe IV. Sex of the specimens was determined by examining the cloacal region for the presence of hemipenes. Geographic coordinates of the areas of its occurrence were recorded with a GPS and mapped with ARC MAP 10. Terminologies for color descriptions follow Poyarkov et al. (2019).

***Lipinia macrotympanum* (Stoliczka, 1873)**  
(Figs 1–2)

*Mocoa macrotympanum* Stoliczka, 1873  
*Lygosoma macrotympanum* – Boulenger (1890)  
*Leioloopisma macrotympanum* – Smith (1935)  
*Lygosoma quadriplacatum* (non Peters, 1867) – Biswas & Sanyal (1977)  
*Lipinia macrotympanum* – Greer (1974); Das (1997); Das & Austin (2007)  
*Lipinia macrotympana* – Das (1999) sic.

INDIA – 1 adult male (ZSI/ANRC/T/3709); Shastri Nagar; 6.810° N, 93.892° E; 37 m a.s.l.; G. Gokulakrishnan leg. 11 Oct. 2015. 1 adult male (ZSI/ANRC/T/4330); Galathea; 6.8216° N, 93.8673° E; 37 m a.s.l.; G. Gokulakrishnan leg. 20 Nov. 2016.

*L. macrotympanum* is a small species of *Lipinia* from the Nicobar Islands, that can be diagnosed and characterized by: the presence of a large and exposed tympanic membrane; 7 supralabials; 6 infralabials; presence of a large, transparent disc on the lower eyelid; a single broad prefrontal with a median constriction or two separated prefrontals (*fide* Das, 1997); two, nearly equal sized loreals; a single, undivided frontoparietal; 4 supraoculars; a pair of enlarged preanal scales; 21–23 midbody scale rows; 51–53 paravertebrals; 60–62 ventrals; 16–17 subdigital lamellae under toe IV; SVL 36.5–45 mm; tail 43.9–50 mm; dorsal coloration of black with three yellow longitudinal stripes from snout to vent; tail red or reddish brown (based on the collected specimens and Das, 1997).

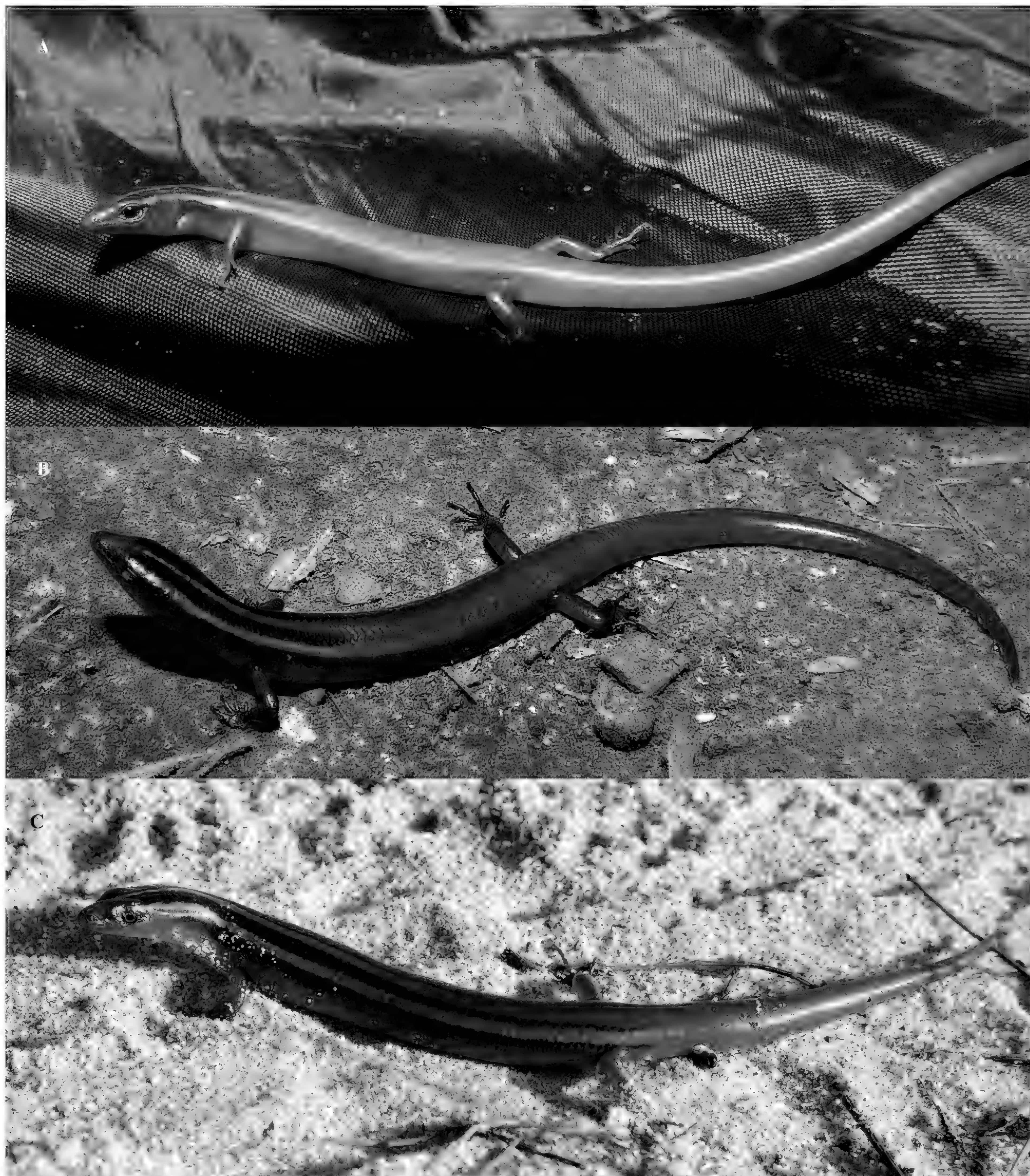
The newly collected material, two adult males, measure 38.5–39.6 mm SVL; overall habitus slender and elongated. Head short (HL:SVL 0.12); slightly longer than broad (HL:HW 1.02) with a pointed snout tip in both dorsal and lateral views. Nostrils located laterally, closer to the snout tip than to the eyes (EN:ES 0.7). Rostral visible from above; frontonasal broader than long; prefrontal single in ZSI/ANRC/T/3709; ‘8’ shaped, with a median constriction; two small rhomboidal prefrontals, in contact with each other in ZSI/ANRC/T/4330; frontal wedge shaped; frontoparietal single; interparietal fairly elongated; parietals large; in contact with each other; three pairs of broad nuchals present. Paravertebrals slightly broader than the adjacent body scales. Supraoculars four; third largest; lower eyelid with a transparent disc. Loreals two, trapezoidal in shape and nearly of equal size. Temporals large and smooth. Supralabials 7, 6<sup>th</sup> largest; infralabials 6. Tympanic opening large (TYD:ED 0.66) with a visible eardrum, lacking auricular lobules. Mental semicircular; a single large postmental. Anterior chin-shields in contact with each other; posterior chin-shields separated by a single scale. Midbody scale rows 21–23; smooth. Limbs fairly well developed. Relative length of fingers IV>III>II>V>I. Thigh short (FEL:SVL 0.12); tibia as

**Table 1.** Measurements of the examined *Lipinia macrotympanum* specimens compared with literature.

	ZSI/ANRC/ T/ 4330	ZSI/ANRC/ T/ 3709	Das (1997)
Total Length	92.5	89.8	80.4–91.7
SVL	38.7	37.6	36.5–38.1
TL	53.8	52.2	43.9–53.6
AG	24	23.8	20.2–21.3
HL	4.8	4.7	5.8
HW	4.7	4.6	3.7–3.9
ES	2.9	2.7	2.7–2.9
EN	2	1.9	1.5–1.6
NS	1.3	1.1	–
ED	1.8	1.7	1.3–1.8
TYD	1.2	1.1	1.0–1.1
IN	1.5	1.4	1.3–1.7
IO	4	3.8	1.3–1.7
FLL	10.11	9.8	8.4
FEL	4.6	4.5	–
TBL	4.6	4.5	–
Paravertebrals	51	53	–
Ventrals	60	62	62
MSR	23	21	21–23
T4 lamellae	15	16	15–17
NU	6	6	6

long as thighs (FEL:TBL 1.0); foot slightly longer. Toe IV longest; relative length of toes IV >III>V >II>I; toe IV with 15 smooth subdigital lamellae; claws long and protruding. Measurements of the material studied are presented in table 1.

Dorsal coloration black to dark brown anteriorly; fading posteriorly to light brown with a reddish brown tail in ZSI/ANRC/T/3709. Dorsum with three yellow stripes; the mid dorsal light stripe (MDLS) originating from the snout and broadening as it proceeds posteriorly towards the sacrum. The other specimen, ZSI/ANRC/T/4330 was golden brown overall, with just the head and neck bearing the dark coloration. Two paravertebral dark stripes (PVDS) commencing from post ocular region, continuing till the sacrum, beyond which it merges with the uniformly reddish brown coloured tail; stripes not discernible posteriorly. Lateral body and limbs orange colored. Lateral dark stripe (LDS) and ventrolateral dark stripes (VLDS) absent. Dark temporal marking (DTM) present in all the samples. Venter uniform creamy white. Underside of tail reddish. The coloration of ZSI/ANRC/T/4330

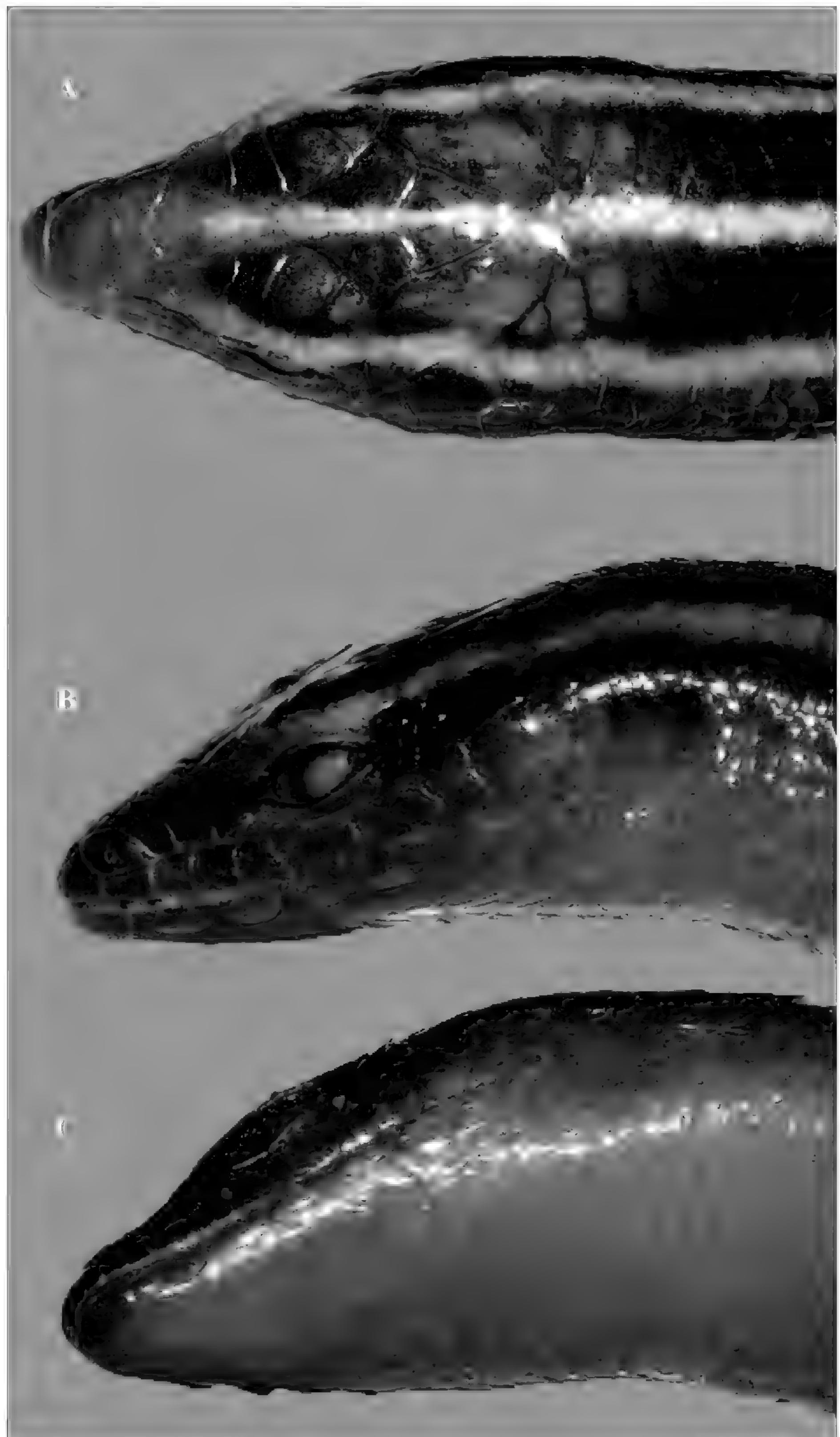


**Fig. 1.** *Lipinia macrotympanum* in life. **A–B.** ZSI/ANRC/T/4330 from Galathea. **C.** ZSI/ANRC/T/3709 from Shastri Nagar.

reported herein has not been known until now. Illustration of this species published by Das (2002) conforms to the coloration of ZSI/ANRC/T/3709.

The first individual recorded from Shastri Nagar (ZSI/ANRC/T/3709) was found near an old building, and was

seen actively moving and approaching the observers after dusk at ca. 19:43 h. The immediate vicinity of this spot is bordered by evergreen forests and a stream to the west and a sandy beach to the south. The second and third individuals from Galathea were observed on the ground;



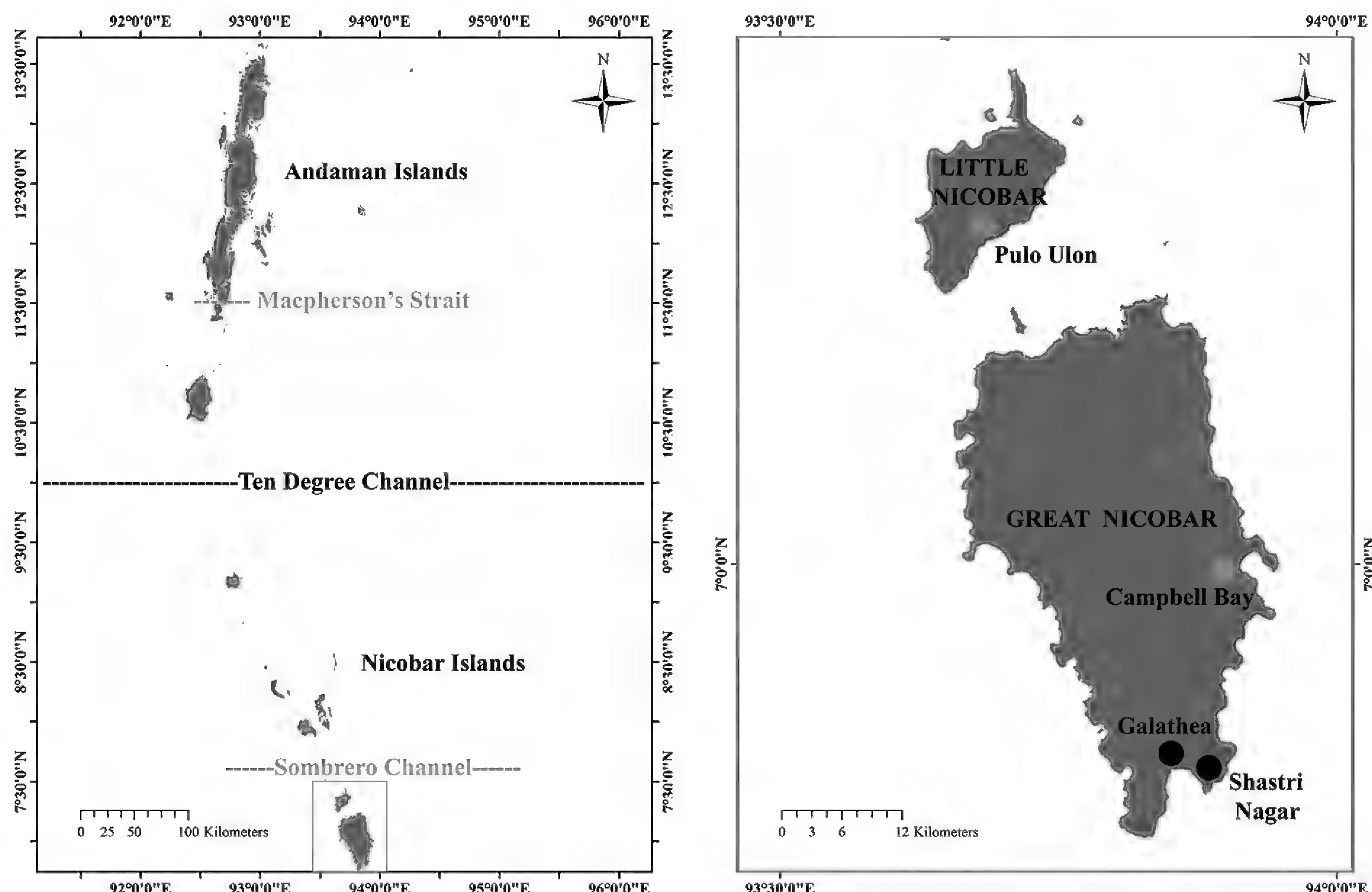
**Fig. 2.** Head of *Lipinia macrotympanum* ZSI/ANRC/T/3709. **A.** Dorsal view. **B.** Lateral view. **C.** Ventral view.

the specimen collected (ZSI/ANRC/T/4330) was found resting under a dry palm leaf post noon at ca. 15:18 h. The third, uncollected individual was found foraging on the ground, at the camp site near the tent in Feb 2016. This region (Galathea) is surrounded by *Casuarina* groves bordering a stretch of a sandy beach near the Galathea River delta. The habitat of this region is generally characterized by reduced canopy cover, low leaf-litter and sandy soil situated close to the sea coast with strand vegetation.

From congeners, *Lipinia macrotympanum* can be distinguished as follows (data for comparison modified from Das & Austin 2007 and Poyarkov et al. 2019): presence of an externally visible tympanic membrane (vs. hidden in *L. sekayuensis*; *L. inexpectata* and *L. surda*); dorsum with a pattern of longitudinal stripes (vs. absent in *L. sur-*

*da* and faint stripes in *L. sekayuensis*); 16–17 subdigital lamellae under toe IV (vs. 19–23 in *L. albodorsalis*; 18–25 in *L. infralineolata*; 7–10 in *L. leptosoma*; 19 in *L. longiceps*; 21 in *L. miangensis*; 20–21 in *L. occidentalis*; 24–31 in *L. pulchella*; 22 in *L. pulchra*; 18–21 in *L. rabori*; 18 in *L. relicta*; 22–26 in *L. rouxi*; 19–21 in *L. semperi*; 18–22 in *L. septentrionalis*; 19–21 in *L. venemai*; 25 in *L. vittigera*. Three pairs of nuchals present in *L. macrotympanum* (vs. five pairs in *L. cheesmanae*; 2 pairs in *L. inexpectata*); 21–23 midbody scalarows in *L. macrotympanum* (vs. 22–25 in *L. albodorsalis*, *L. auriculata*, 28 in *L. cheesmanae*, 22–26 in *L. leptosoma*, 24 in *L. longiceps* and *L. miangensis*; 24–28 in *L. noctua*; 24–25 in *L. notolineata*; 24–26 in *L. occidentalis*; 22–26 in *L. pulchella*; 24 in *L. pulchra*; 22–28 in *L. rouxi*; 24–26 in *L. septentrionalis*; 24–26 in *L. venemai*; 28 in *L. vittigera* and *L. vassilievi*; 32 in *L. vulcania*; 24 in *L. zamboangensis*; 28–32 in *L. microcercus* and *L. trivittata*.

*Lipinia macrotympanum* has been one of the most poorly known skink species in the Andaman and Nicobar archipelago, which has been recorded only three times since its description in 1873 (Biswas & Sanyal 1977; Das 1997; Rangasamy et al., 2014). Other herpetofaunal surveys (e.g., Vijayakumar 2005, Harikriahnan et al. 2008, 2014) did not record *L. macrotympanum*. The present observations of *L. macrotympanum* reported here are from the southern extremity of Great Nicobar and are at least 35–40 km south of the closest previously known locality from Great Nicobar (i.e., Campbell Bay *fide* Biswas & Sanyal, 1977) thereby extending its distribution range further south. Das (1997) stated that the individual recorded by him was also seen on the sand, moving with great agility. *L. macrotympanum* being active after dusk reported here is novel information as earlier authors have all recorded it during the day time. The individuals reported here were also seen on the ground as reported by Das (1997). The type locality of this species, Macpherson's Strait lies between the southern tip of South Andaman and Rutland Island. There have been no reports of this species from any of the islands in the Andaman archipelago since its original description. All of the subsequent reports (Biswas & Sanyal 1977, Das 1997, this work) have been from the southern group of islands in the Nicobar archipelago, i.e., Little and Great Nicobar Islands, situated to the south of the Sombrero Channel. The Andaman Islands, situated in the north of the ten-degree channel are biogeographically different from those of the Nicobar Islands and their fauna show a more Indo-Chinese faunal affinity (Das 1999). It is speculated that the type locality of *L. macrotympanum* mentioned by Stoliczka (1873) could be inaccurate and the species may not actually occur in the Andaman Islands. The possible absence of *Lipinia* in the Andaman Islands is further supported by the fact that it has never been recorded from islands of the Andaman archipelago after the report by Stoliczka (1873) although several herpetofaunal surveys



**Fig. 3.** Type locality (Macpherson's Strait) of *Lipinia macrotypanum*: Literature records (red dots) and new locality records (black dots) in Great and Little Nicobar Islands.

have been and are still being carried out in the Andaman archipelago (e.g., Das, 1999; Harikrishnan et al., 2014; Rangasamy et al., 2014; pers. obs.). Similarly, there have been some intriguing records of certain species which were mentioned in older literature from certain regions, but have never been recorded from such localities subsequently. Examples include *Lycodon tiwarii* from Mayabunder, North Andaman, *Oligodon woodmasoni* from Andamans for both of which, confirmed current records are only from the Nicobar Islands (Vijayakumar & David 2006) and *Hemidactylus platyurus* which was once reported from Great Nicobar (Tiwari & Biswas 1973) but is presently known from the Andaman Islands, and not Nicobars.

Based on the observations reported until now, we recommend to regard *L. macrotypanum* as an endangered species based on the criteria B1 (Extent of occurrence less than 5000 km<sup>2</sup>) and B2 (Area of occupancy less than 500 km<sup>2</sup>) of IUCN. Further studies are required to better understand the ecology of this poorly known, narrowly endemic species.

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## Research article

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# The first record of the genus *Fulvius* Stål, 1862 (Heteroptera: Miridae: Cylapinae) from continental China with description of a new species

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**Abstract.** A new species of the genus *Fulvius* Stål, 1862 (Heteroptera: Miridae: Cylapinae: Fulviini) is described based on a couple of specimens collected in Yunnan Province in SW China. The genus is also reported from continental China for the first time. Detailed illustrations of the tarsi, the distribution of trichobothria on the metafemur and male genitalia are given, and an image of the dorsal habitus is presented.

**Key words.** Asia, biodiversity, new species, plant bugs, taxonomy, true bugs, Yunnan Province.

## INTRODUCTION

*Fulvius* Stål, 1862 is known as one of the most speciose genera within the subfamily Cylapinae, with more than 80 valid species worldwide (Wolski et al. 2018). Nevertheless, the state of the knowledge about this genus is probably far from being complete. Most species occur in the New World and the Afrotropical and Oriental Regions. Only a few species are known from the Australian Region and very few have been described from the Palearctic Region (Gorczyca 2006). Little is known about the biology of the congeners. While most members of *Fulvius* are frequently collected using UV light traps, some of them are saproxylic and have been found on fallen decaying wood or often on fungi, sucking the fungal hyphae (Gossner & Damken 2018; Kim et al. 2019). On the other hand, some of them are carnivorous (Yasunaga & Miyamoto 2006; Pluot-Sigwalt & Cherot 2013).

While examining some material housed in the Department of Entomology at the National Museum of Natural History, Prague, Czech Republic, two specimens of the genus *Fulvius*, collected in Yunnan Province of SW China were found. These were confirmed as representing an undescribed species of *Fulvius*, which is described in this paper. This discovery also reveals the first distributional record for the genus from continental China.

## MATERIAL AND METHODS

The specimens were imaged using the following equipment: a Leica M205C stereo microscope with a Leica DFC495 digital camera and Leica application suite 4.9.0 software; a Leica DM 3000 upright light microscope with a Leica MC 190 HD digital camera and Leica Application Suite 4.12.0 software and an Olympus upright light microscope with a Canon EOS 750D digital camera. SEM photographs were obtained using a Phenom XL field emission scanning electron microscope at 10 and 15 kV accelerating voltages using a BackScatter Detector (BSD). Measurements were taken with Leica application suite 4.9.0 software and are presented in millimetres (mm). The total body length is defined as the length from the apex of the clypeus to the posterior margin of the membrane. The measured body parts were defined in Wolski (2015). Genitalia were kept in 10% KOH solution before dissection, and the female genitalia were stained with chlorazol-black. The terminology of the male genitalic structures follows Kerzhner & Konstantinov (1999), Konstantinov (2003) and Cassis (2008), and the terminology of the female genitalia follows Davis (1955), Sadowska-Woda et al. (2006) and Pluot-Sigwalt & Matocq (2017).

## RESULTS

### Taxonomy

#### *Fulvius yunnanicus* sp. nov.

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### Type material

**Holotype** (♂). China, Yunnan Prov., 1.8 km W Zizhi vill., 2.vii. 2016, 25°44.7' N, 98°33.6' E, 2005 m a.s.l., from large dead tree stumps, J. Hájek & J. Růžička leg.; collection of the National Museum Praha, Czech Republic. **Paratype** (♀). Same data as for holotype; both are preserved in the Department of Entomology, the National Museum, Prague, Czech Republic.

**Diagnosis.** The new species belongs to the *anthocoroides*-group (see discussion below) and can be distinguished from other members of the group by the following combination of characters: pronotum dark brown with three brown longitudinal stripes spanning its whole length; antennal segments I–II dark brown to black with contrastingly yellow apical 1/4; legs entirely brown (Fig. 1); endosoma with large, oval sclerotized lobe apically (Fig. 4C); bursa copulatrix with ring-like sclerite situated near base of the seminal depository; sclerotized rings weakly developed (Fig. 5A).

Most similar to *F. mateusi* Sadowska-Woda & Gorczyca, 2008, *F. nigricornis* Poppius, 1909 and *F. tagalicus* Poppius, 1914 in sharing the uniformly coloured hemelytron. *F. yunnanicus* can, however, be easily distinguished from these species by having the pronotum with brown, longitudinal patches along entire length (Fig. 1) and the presence of the oval sclerotized lobe in the endosoma (Fig. 4C) (see remarks below).

## DESCRIPTION

### Male

**Colouration** (Fig. 1). Dorsum, pale brown with darker, mostly dark brown areas.

**Head.** Dark brown, tinged with yellow, first and second segment vary from dark brown to nearly black, second segment contrastingly yellow apically, third and fourth segments dark brown; labium brown, last segment dark brown.

**Thorax.** *Pronotal collar*. Brown. *Pronotum*. Dark brown with two brown longitudinal stripes. *Mesoscutum and scutellum*. Dark brown, scutellum pale at apex. *Thoracic pleura*. Proepimeron, mesepisternum and meseppimeron dark brown almost black. *Hemelytron*. Pale brown, partly translucent, exocorium slightly tinged with red; clavus pale brown at base, dark brown in apical part with large dark brown and reddish patch contiguous



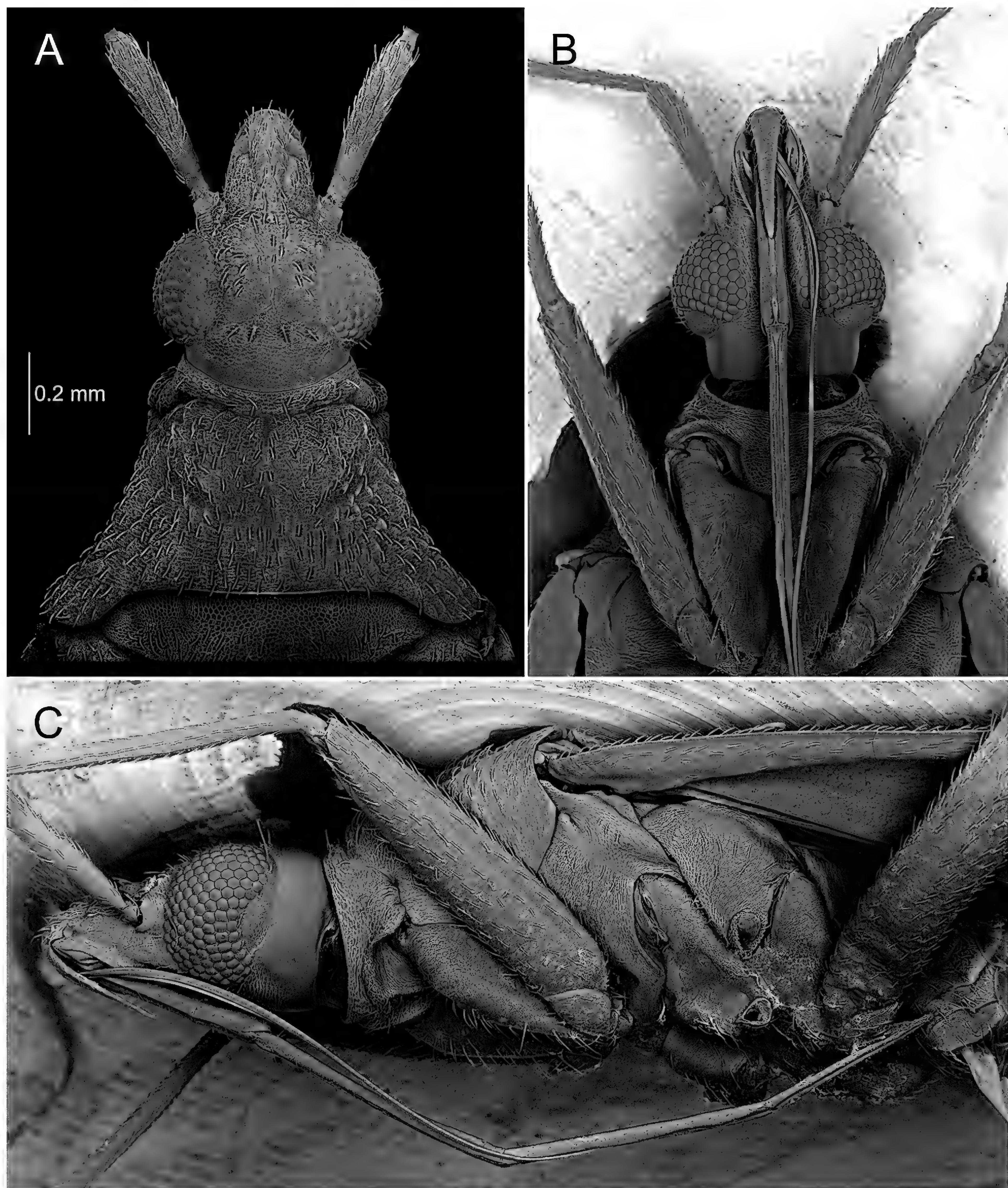
**Fig. 1.** *Fulvius yunnanicus* sp. nov., holotype, dorsal view.

with clavus and membrane; cuneus dark brown, paler at apex; membrane grey, venation dark grey. Large areolar cell triangular; small areolar cell very small. *Legs*. Pale brown, femora in apical part slightly tinged with red.

**Abdomen.** Chestnut to dark brown.

**Structure, texture and vestiture.** Dorsum matte, covered with fine, pale, very short closely fitting, scale-like setae.

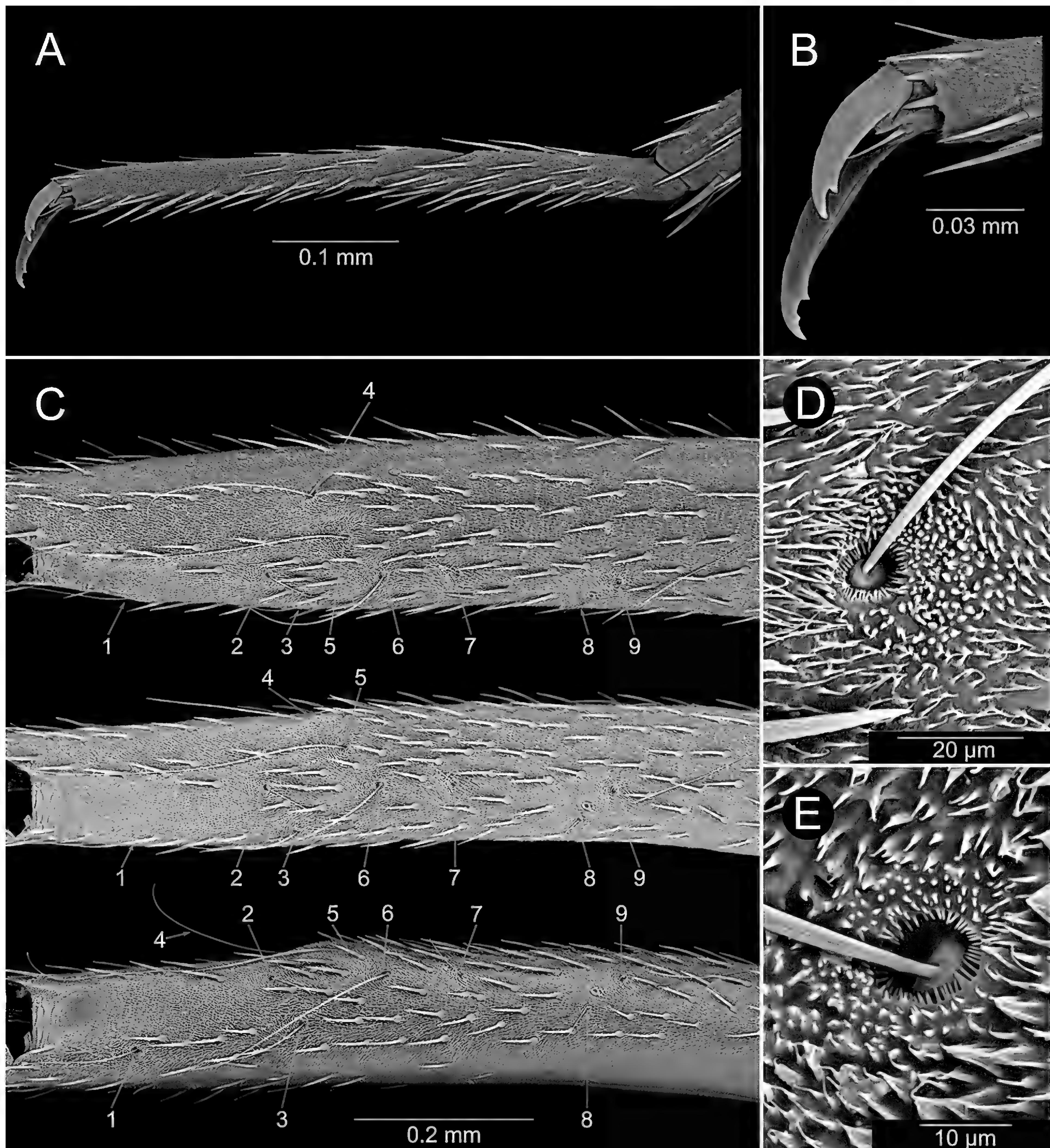
**Head.** Eyes contiguous with pronotal collar; first and second antennal segments covered with dark, short setae (Figs 1–2); second segment slightly thickened towards apex; third and fourth segments very thin, covered with pale, long, protruding setae. Labium reaches beyond metacoxae (Fig. 2C).



**Fig. 2.** *Fulvius yunnanicus* sp. nov., holotype, front part of the body. **A.** Dorsal view; **B.** Ventral view; **C.** Lateral view.

**Thorax. Pronotum.** Anterior lobe of pronotum only slightly convex with thin longitudinal sulcus medially (Fig. 2A).

**Legs.** Relatively long (Fig. 1), covered with very short setae, much shorter than diameter of tibiae; metafemora with nine trichobothria (Fig. 3C–E); tarsi two-segment-

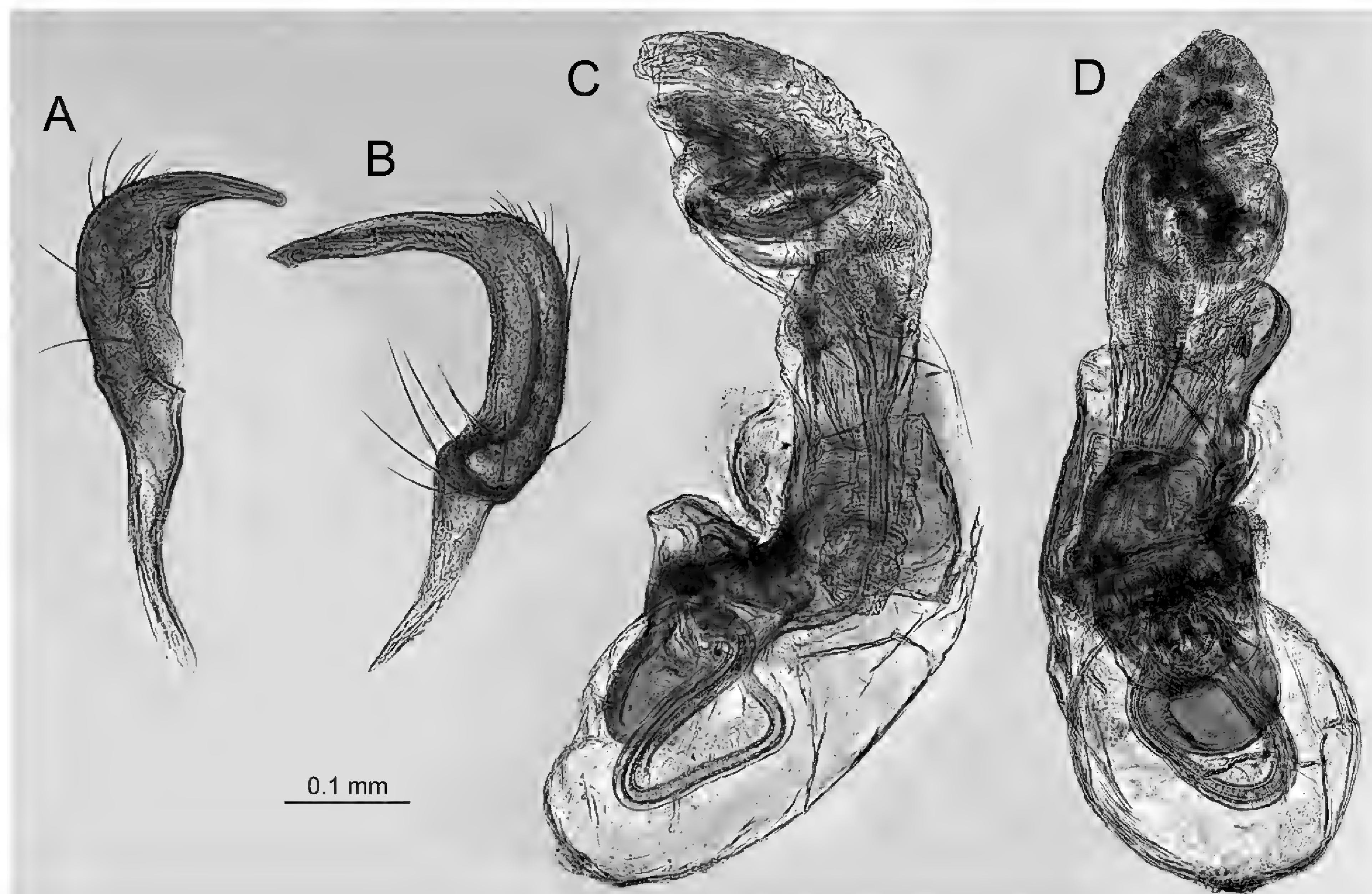


**Fig. 3.** *Fulvius yunnanicus* sp. nov. **A.** Paratype, metatarsus; **B.** Claws in details; **C.** Holotype, distribution of trichobothria on the metafemur; **D–E.** Details of the structure of the trichobothria.

ed, second segment not divided; claws with distinct subapical tooth (Fig. 3AB). *Hemelytron*. Major cell triangular, minor cell very small.

**Male genitalia** (Fig. 4). Typical of *anthocoroides* group (Gorczyca 2002; Sadowska-Woda et al. 2008; Wolski et al. 2018, also see discussion below). *Right paramere*. Apical process thin and relatively long; spine

on inner surface of paramere body indistinct. *Aedeagus*. Sclerotized part of seminal duct broadened apically; endosoma with strongly developed, arcuate sclerite and large, elliptical sclerotised lobe on apical half.



**Fig. 4.** *Fulvius yunnanicus* sp. nov., holotype, male genitalia. A. Right paramere; B. Left paramere; C. Lateral view of endosoma; D. Dorsal view.

#### Female

Similar to male in colouration, structure, texture and vestiture.

**Female genitalia** (Fig. 5). Genital chamber (or bursa copulatrix) rounded; lateral oviducts short, slightly broad apically, ring-like sclerite near basal part of seminal depository rounded, protruding, prominent, broadly developed; sclerotized ring situated laterally, indistinct; posterior wall with wrinkled interramal sclerite; membranous structure present between gonapophysis I.

**Measurements** (mm). ♂/♀ (holotype measurements first)

**Body.** Length 4.09/4.10, width 1.36/1.36.

**Head.** Length of head 0.79/0.79, width 0.61/0.66, dorsal width of eye 0.17/0.18, width of vertex 0.27/0.28.

**Antenna.** Length of segment I 0.51/0.52, II 1.14/1.19, III 0.51/0.51, IV 0.50/ missing in ♀.

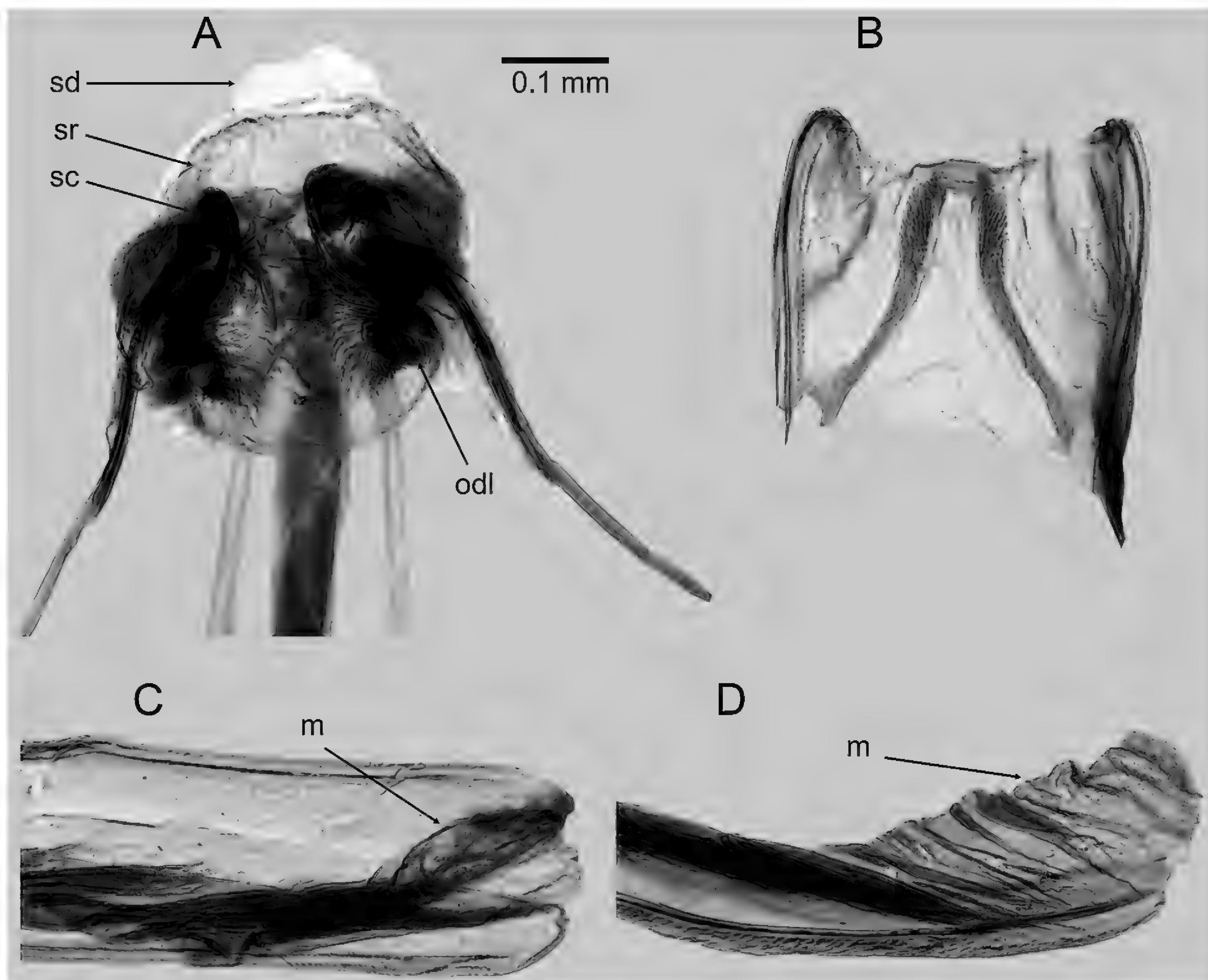
**Labium** (unmeasurable in specimens examined).

**Pronotum.** Length 0.61/0.63, length of lateral margins 0.67/0.77, length of anterior margins 0.51/0.52, length of posterior margins 1.14/1.37

**Distribution.** China, Yunnan Province (Fig. 6).

**Etymology.** The specific epithet refers to the Chinese province Yunnan where the specimens were collected.

**Remarks.** Gorczyca (2002), Sadowska-Woda et al. (2008) and Wolski et al. (2018) summarized the morphological characters that define the three species groups that are currently found in the genus *Fulvius* Stål, 1862: the *anthocoroides*, *bifenestratus*, and *bisbistillatus* groups. The presented new species can easily be classified as a member of the *anthocoroides* group as it has the following characters: a) dorsum matte, covered with uniformly distributed setae (Fig. 1; Wolski et al. 2018: figs 41–44); b) second tarsomere not subdivided medially, subapical tooth present (Fig. 3A–B; Wolski et al. 2018: figs 45–46); c) the aperture of the pygophore is oriented posteriorly, with long dorsal wall (Wolski et al. 2018: figs 47–48); d) the parameres are similar in size, the right paramere has a relatively thick paramere body and a thin and short apical process; the left paramere has a relatively long apical process with a subapical incision (Fig 4A–B; Carvalho and Lorenzato 1978: figs 56–57, 68–69; Gorczyca 2002: figs 1–4; Pluot-Sigwalt & Chérot 2013: fig. 4B–C; Yasunaga 2000: figs 23–24, 28–29; Yasunaga and Wolski 2017: figs 3A–B); e) endosoma with sclerites or sclerotized appendages; the sclerotized portion of the seminal

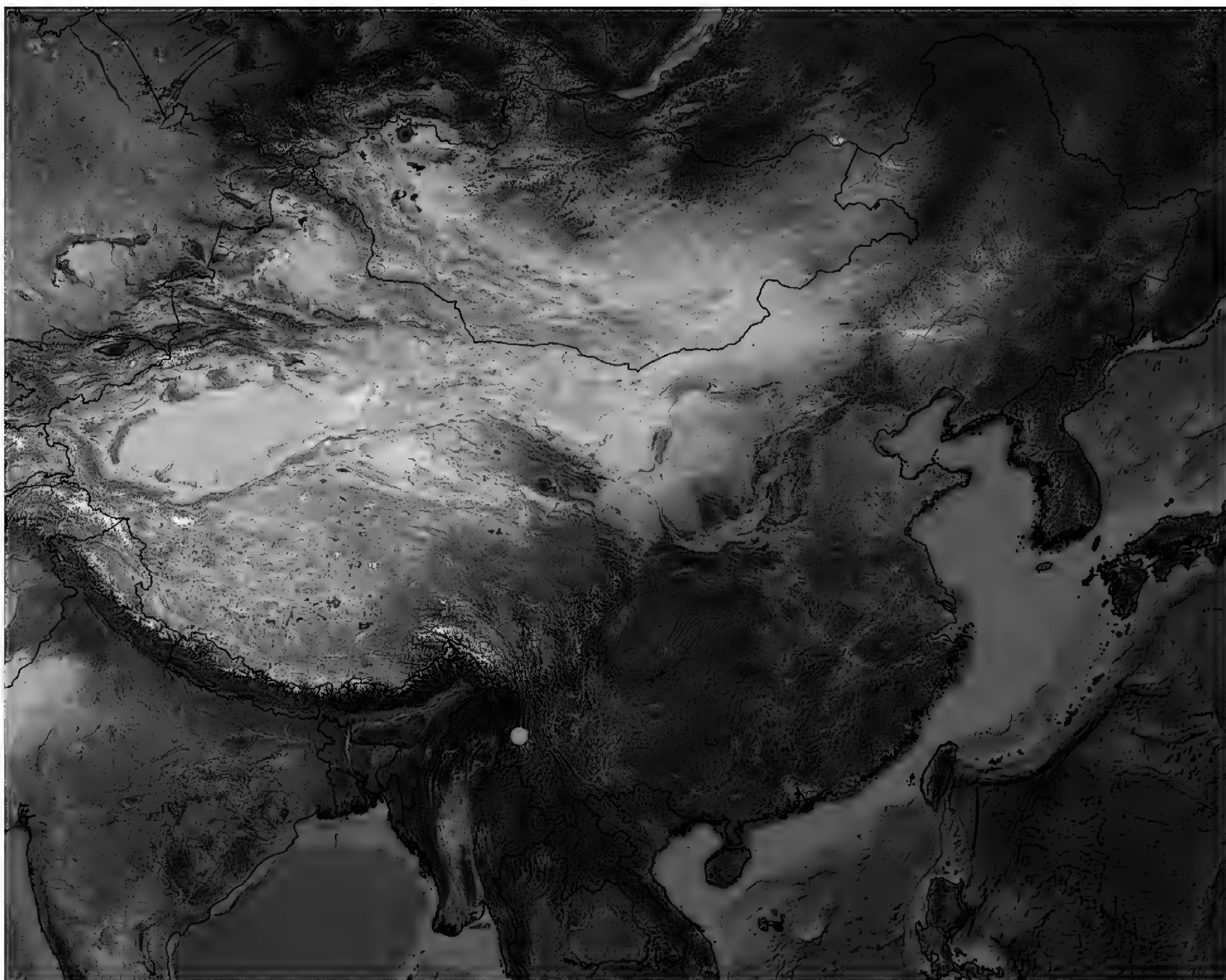


**Fig. 5.** *Fulvius yunnanicus* sp. nov., paratype, female genitalia: **A–B**. Genital chamber (or bursa copulatrix). **A**. Dorsal view; **B**. Posterior wall, anterior view; **C**. Gonapophysis I and adjacent structures, dorsal view; **D**. Gonapophysis I and adjacent structures, right lateral view. *m* = membranous structure adjunct to gonapophysis I; *odl* = lateral oviduct; *sc* = ring-like sclerite near basal part of seminal depository; *sd* = seminal depository; *sr* = sclerotized ring.

duct is well developed, long and tubular (Fig. 4C–D; Carvalho & Lorenzato 1978: fig. 55; Pluot-Sigwalt & Chérot 2013: fig. 4A; Yasunaga 2000: fig. 30; Yasunaga & Wolski 2017: fig. 3C) and f) a membranous structure is present between the gonapophysis I (Fig. 5C–D; Sadowska-Woda et al. 2008).

Within the *anthocoroides* group *F. yunnanicus* sp. nov. is most similar to the Oriental *F. mateusi* Sadowska-Woda & Gorczyca, 2008, *F. nigricornis* Poppius, 1909 and *F. tagalicus* Poppius, 1914. All these species have uniformly coloured hemelytron (e.g., Yasunaga 2000: fig. 19), not having any pale patch basally as it is found for example in *F. anthocoroides* (Reuter, 1875), *F. dimidiatus* (Poppius, 1909) or *F. ussuriensis* Kerzhner, 1973

(e.g., Yasunaga 2000: figs 18, 22; Yasunaga & Wolski 2017: fig. 2C). The present new species can, however, be easily distinguished from *F. mateusi*, *F. nigricornis* and *F. tagalicus* by having the pronotum with brown, longitudinal patches along entire length (Fig. 1) and the presence of the oval sclerotized lobe in the endosoma (Fig. 4C). From *F. mateusi* *F. yunnanicus* sp. nov. can be also distinguished by the antennal segment II with contrastingly yellow annulation apically (Fig. 1) (antennal segment II is uniformly dark brown in *F. mateusi*) and the presence of the ring-like sclerite near base of the seminal depository (Fig. 5A) which is lacking in *F. mateusi* (Sadowska-Woda & Gorczyca 2008: fig. 6).



**Fig. 6.** Distribution of *Fulvius yunnanicus* sp. nov.

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## Research article

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# Status and distribution of the little-known and elusive Nicobarese worm lizard *Dibamus nicobaricum* (Fitzinger in Steindachner, 1867) (Squamata: Dibamidae)

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**Abstract.** Field surveys were carried out to record the elusive and little-known fossorial Nicobarese worm lizard *Dibamus nicobaricum* (Fitzinger in Steindachner, 1867) on seven of the 23 islands of the Nicobar archipelago. It was recorded from three new localities, two in Great Nicobar and the other from Teressa Island, extending the northern and southern boundaries of its distribution significantly. One of the individuals, a subadult male recorded during this study happens to be the smallest one ever recorded, measuring just 70 mm SVL. A predictive distribution model was developed based on the geo-coordinates of its occurrence with a reliable prediction of 25–100% probability on islands of the central and southern group of the Nicobar archipelago, diminishing to 12–25% on Car Nicobar, situated to the north. The Area Under the Curve (AUC) of the model was 0.907, indicating a reliable prediction. Status of *D. nicobaricum* was assessed for the first time as per the IUCN guidelines which reveal that it has to be considered as an endangered species based on its narrow distribution range.

**Key words.** Nicobar worm-lizard, *Dibamus nicobaricum*, distribution, status, MAXENT model.

## INTRODUCTION

Dibamids are one among the oldest living group of squamate reptiles (Pyron et al. 2013). The genus *Dibamus* Duméril and Bibron, 1839 currently has 24 species (Uetz et al. 2020) of which *Dibamus nicobaricum* (Fitzinger in Steindachner, 1867) is one among the earliest known species. The holotype was collected by G.R. Frauenfeld from “Nicobars” during the global voyage of the Austrian Frigate “SMS Novara” and described originally by Fitzinger in Steindachner (1867) as *Rhinophidion nicobaricum*. In the same publication, Steindachner (1867) reported on similarities between *Rhinophidion* Fitzinger, 1967 and *Typhloscincus* Peters, 1864 resulting in the transfer of the species *nicobaricum* to *Typhloscincus*. Later Stoliczka (1873) attributed this species to the genus *Dibamus* Duméril & Bibron, 1839 where it is currently placed. Dibamids, being fossorial and small bodied organisms, have remained extremely elusive and hence, not many records of most species exist.

The Nicobar worm-lizard *Dibamus nicobaricum* has been reported only a very few times since its original description. Stoliczka (1873) considered *Dibamus nicobaricum* to be a synonym of *Typhloscincus Martensii* Peters,

1864 from Ternate Island, Indonesia (now *Dibamus novaeguineae*). Annandale (1904), Humayun Abdulai (*fide* Das 1996), Biswas and Sanyal (1977), and Das (1996) are some of the authors who recorded this species from Great Nicobar and Biswas and Sanyal (1980) recorded it from Camorta in the Central group of Nicobar Islands based on a single adult female specimen. Biswas and Sanyal (1977) reported on morphological and sexual variation within this species based on the examination of three specimens. Based on an examination of the above material and his own collection from Shompen Hut in Great Nicobar, Das (1996) resurrected *D. nicobaricum* from the synonymy of *Dibamus novaeguineae* to a distinct and valid species. Also, he considered the record from Camorta to be doubtful (Das 1996: 160). Although this species was discovered about a century and a half ago there is an apparent lack of knowledge concerning its biology, population structure and distribution within the Nicobar archipelago. This deficiency of data also results in the fact that its current status has not yet been assessed according to IUCN criteria yet. Hence, the present study was conducted to determine the status and distribution of the Nicobar worm-lizard *Dibamus nicobaricum*.

**Table 1.** Morphology of two individuals of *D. nicobaricum* recorded during this study (measurements in mm).

Character	individual from Great Nicobar	ZSI/ANRC (T)-7718 (Teressa)
SVL	70	107.6
Tail length	11	24.5
Body Width	2.9	7.4
Tail width	2.2	6.6
Head length	3.3	6.1
Head width	2.8	5.5
Head depth	2.8	4.6
Eye to nostril	2.5	3.5
Number of ventrals	218	183
Number of subcaudals	38	19
Midbody scale rows	23	25
Sex	male	male

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## MATERIAL AND METHODS

Visual encounter survey method (following Crump and Scott, 1994) was employed to collect data on the occurrence of the target species. Islands were selected in such a way that at least one of each subgroup of islands was represented and most of the relatively larger islands (with geographic area  $> 50 \text{ km}^2$ ) were included. The forests were walked by foot and surveys were conducted for one hour duration, wherein specific types of habitats such as evergreen forests, semi evergreen forests, riparian forests, coastal moist deciduous forests and plantations and microhabitats such as leaf-litter, dry stream beds, buttresses and soil under rocks and decayed fallen logs were carefully inspected for the presence of the target species. One of the specimens recorded here from Teressa Island was collected, preserved and deposited at ZSI ANRC (Zoological Survey of India, Andaman and Nicobar Regional Centre), Port Blair. Surveys were conducted both during the day and night time. Logs were turned, leaf litter was disturbed, loose soil was dug and tree buttresses were specifically examined in detail with a flash light to detect the target species. Behavior of the individuals recorded during this study was observed *in situ* following Altmann (1974). Photographic documentation was carried out in the natural habitat, but upon capture. Morphological characters namely, the number of scale-rows at midbody, supralabials, infralabials, number of ventrals and subcaudals were examined with a magnifying lens and the following measurements; snout-vent length (SVL), tail length, body width, tail width, head length, head width, head depth, eye-nostril distance were recorded using vernier calipers to the nearest 0.1 mm. All of the survey locations were marked with a GPS and mapped.

The geo-coordinates of the survey locations were recorded with a Garmin 12 channel GPS and were pooled with those based on literature records; thereby a consolidated set of occurrence points was available to us. This set of GPS points were used in a predictive distribution modeling based on maximum entropy algorithm – MAXENT v. 3.3.3 (Phillips et al. 2006). For making predictions of occurrence of the target species, climatic data were downloaded for the relevant tile from the Worldclim database (Hijmans et al. 2005) and appropriately clipped to the area of interest with DIVA GIS ver. 7.5. Based on these predictions and the geo-coordinates of its occurrence, the possible extent of occurrence and the exact area of occupancy of *D. nicobaricum* was determined (derived from Geocat: <http://geocat.kew.org/>). These were then used as variables in the status assessment as per the IUCN norms version 3.1 (IUCN 2012).

## RESULTS

Three individuals were recorded from new localities during this study; one from Govind Nagar, another from Galathea Bay in Great Nicobar and the third one from Kalasi in Teressa Island. *Dibamus nicobaricum* is recorded from Teressa Island in the central Nicobar Islands for the first time and this also forms the northernmost record of this species. Likewise, the one recorded from Galathea forms the southernmost record. One of the specimens from Govind Nagar (Fig. 1A) recorded during this study is the smallest one ever recorded, measuring just 81 mm, with 70 mm SVL. This specimen is described below in detail.



**Fig. 1.** *Dibamus nicobaricum* recorded during this study in life: A – C: Govind Nagar, D - Galathea, Great Nicobar.

**(a) Morphology (Figs 1–2)**

Body vermiform; head blunt; neck and eyes indistinct; nostrils located towards the snout tip, more ventral than dorsal or lateral in position; rostral large, roughly as broad as long, occupying  $\frac{1}{4}$  of the head length. Frontonasal much broader than long; relatively smaller than frontal. Frontal shield pentagonal with the vertex pointing posteriorly, slightly wider and fairly longer than the frontonasal. Ocular shield horizontally elongate, situated between the edges of frontal and frontonasal shields; eyes located underneath the ocular scales but visible. Postocular single and fairly large. Two distinct supralabials visible on either sides; anterior one much elongated and wedge shaped; posterior relatively shorter and bordering ocular and postocular shields. Mental shield small, bordered by one infralabial (the first) on each side. Body scales smooth, glossy and fairly imbricate. Forelimbs completely absent; two small skin-flaps situated on either sides of the vent (immediately above), indicating the rudiments of hind-limbs. Measurements and pholidosis of the specimens examined are presented in Table 1. Dorsal body unpatterned; uniform reddish brown in colour. Ventral region more pinkish and slightly lighter than the dorsal.

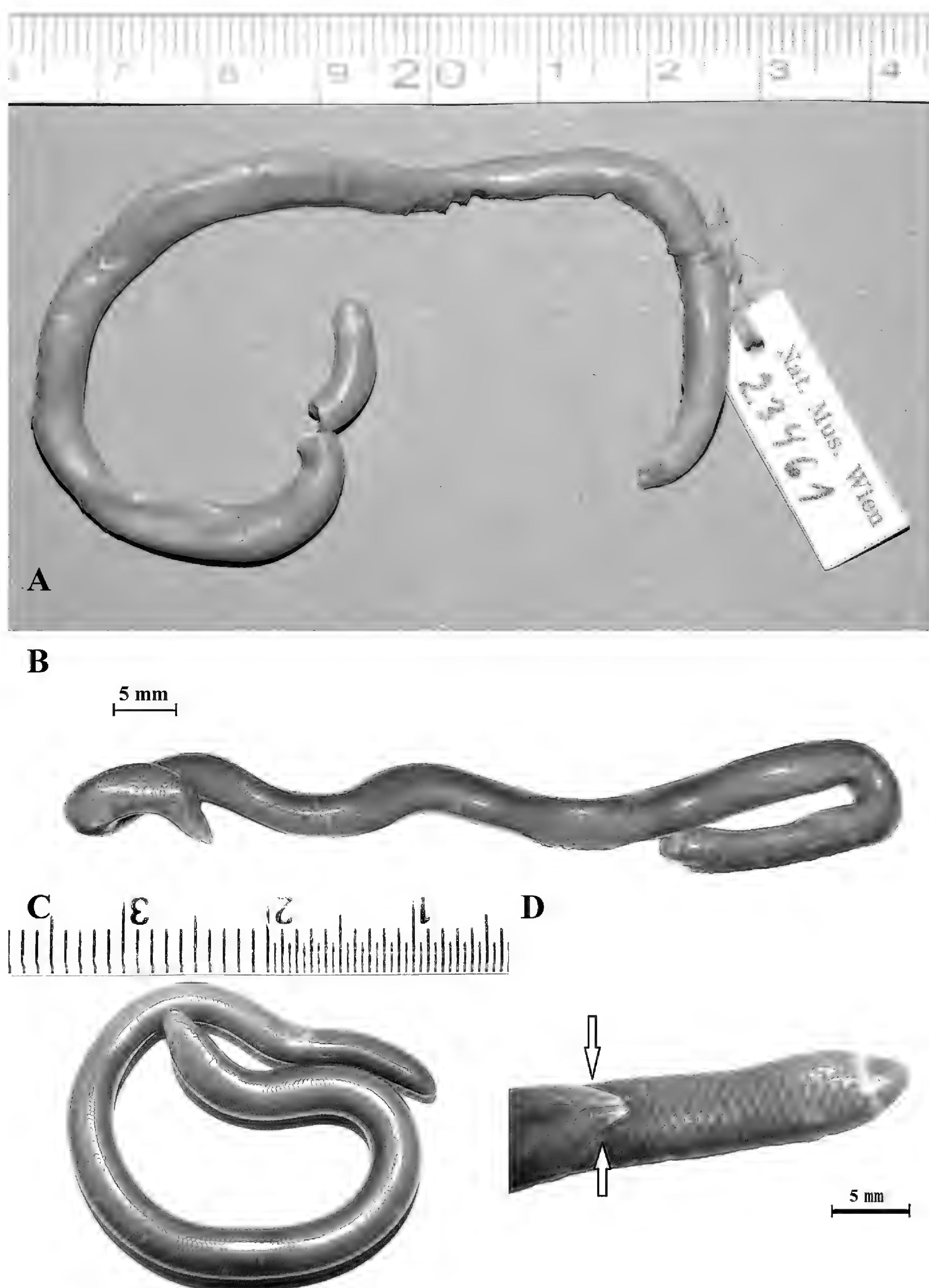
**(b) Distribution (Fig. 3)**

*Dibamus nicobaricum* was recorded from three new localities during the present study, two of which are from Great Nicobar, where it is already known and a first record from Teressa Island. An adult male was recorded from Kalasi in Teressa Island, under the soil at about 5 cm depth in a coconut plantation (under exploitation) and a juvenile male was recorded from under a log in riparian habitat along the banks of a seasonal stream near Govind Nagar, Great Nicobar (Fig. 4). Both these were in dense evergreen forests under thick canopy cover. The third individual was recorded from under a log in a littoral forest in Galathea Bay, Great Nicobar, forming the southernmost record for this species.

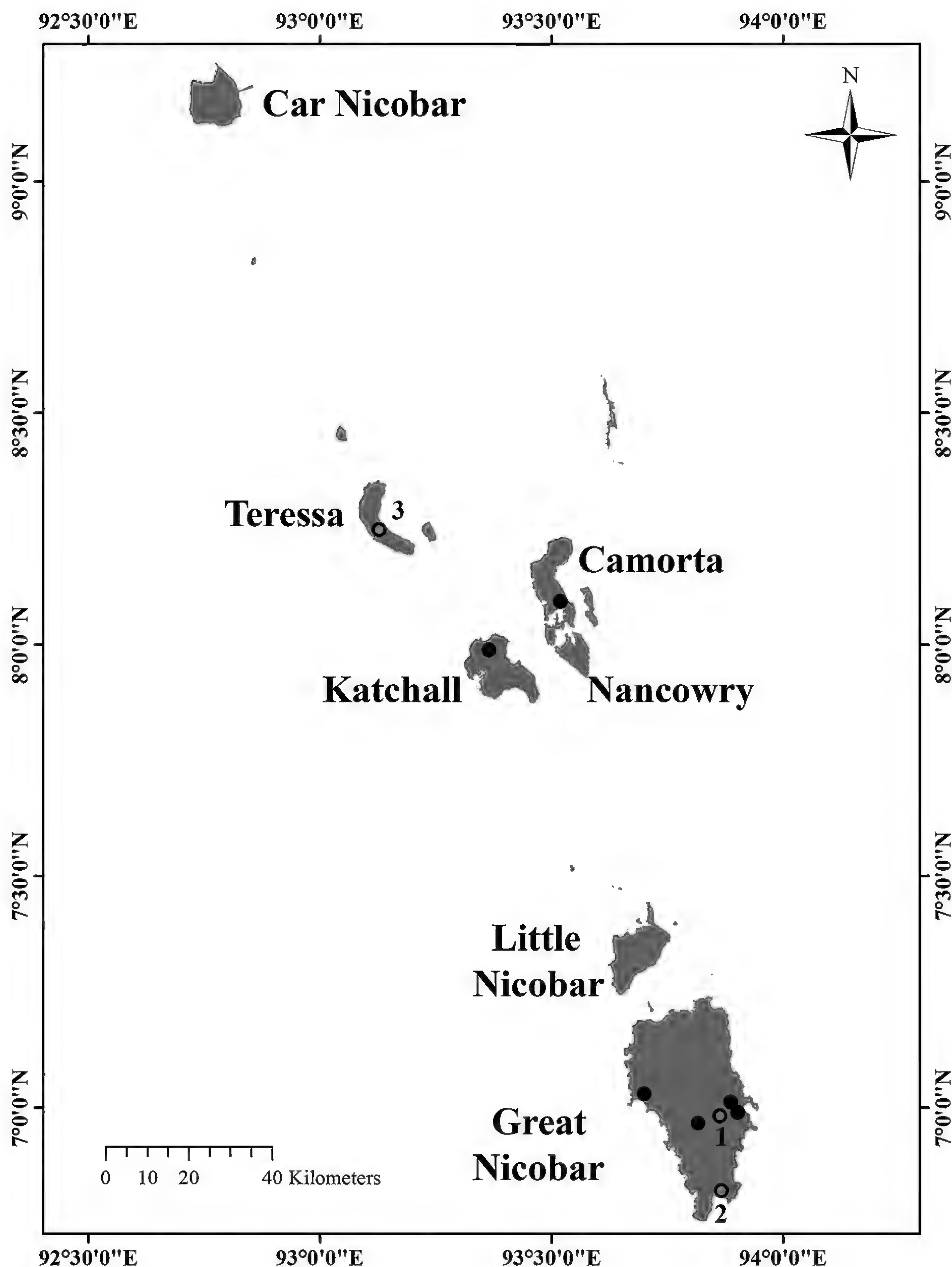
The MAXENT model predicted the distribution of *D. nicobaricum* in Great and Little Nicobar islands with occurrence probabilities ranging from 1 to 0.25 in Great and Little Nicobar islands and islands of the Central group, which diminishes to 0.12–0.25 in Car Nicobar, the northernmost island of the Nicobar archipelago. AUC (area under the receiver operating characteristic curve) value was 0.908 with significant contributions by the following variables: precipitation of the wettest month

**Table 2.** Percentage contribution of bioclimatic and physiographic variables to the model.

Variable	% contribution	Permutation importance
_bio13_29_a	45.8	0
_bio16_29_a	42.5	72.6
_bio17_29_a	4.7	0
_bio6_29_a	4.1	15.7
_bio7_29_a	2.9	11.7
_bio2_29_a	0	0
_bio1_29_a	0	0
_bio19_29_a	0	0
_bio18_29_a	0	0
_bio15_29_a	0	0
_bio14_29_a	0	0
_bio9_29_a	0	0
_bio8_29_a	0	0
_bio5_29_a	0	0
_bio4_29_a	0	0
_bio3_29_a	0	0
_bio12_29_a	0	0
_bio11_29_a	0	0
_bio10_29_a	0	0
_alt_29_a	0	0



**Fig. 2.** *Dibamus nicobaricum*. **A.** Holotype of *D. nicobaricum* NMW 23461 (courtesy: Gernot Vogel). **B.** ZSI/ANRC (T)-7718 collected from Teressa, central Nicobars. **C.** Ventral view of an individual from Great Nicobar, in life. **D.** Ventral view of tail, showing hindlimb rudiments (arrows).



**Fig. 3.** Distribution records of *Dibamus nicobaricum* in the Nicobar archipelago. New records from this study marked in red.



**Fig. 4.** Habitat of *Dibamus nicobaricum* near Govind Nagar in Great Nicobar.

(45.8%), precipitation of wettest quarter (42.5%), precipitation of the driest month (4.7%), minimum temperature of coldest month (4.1%) and annual temperature range (2.9%). The other factors pertaining to climate and altitude did not have any influence on the prediction (Table 2, Figs 5–7).

### (c) Behaviour

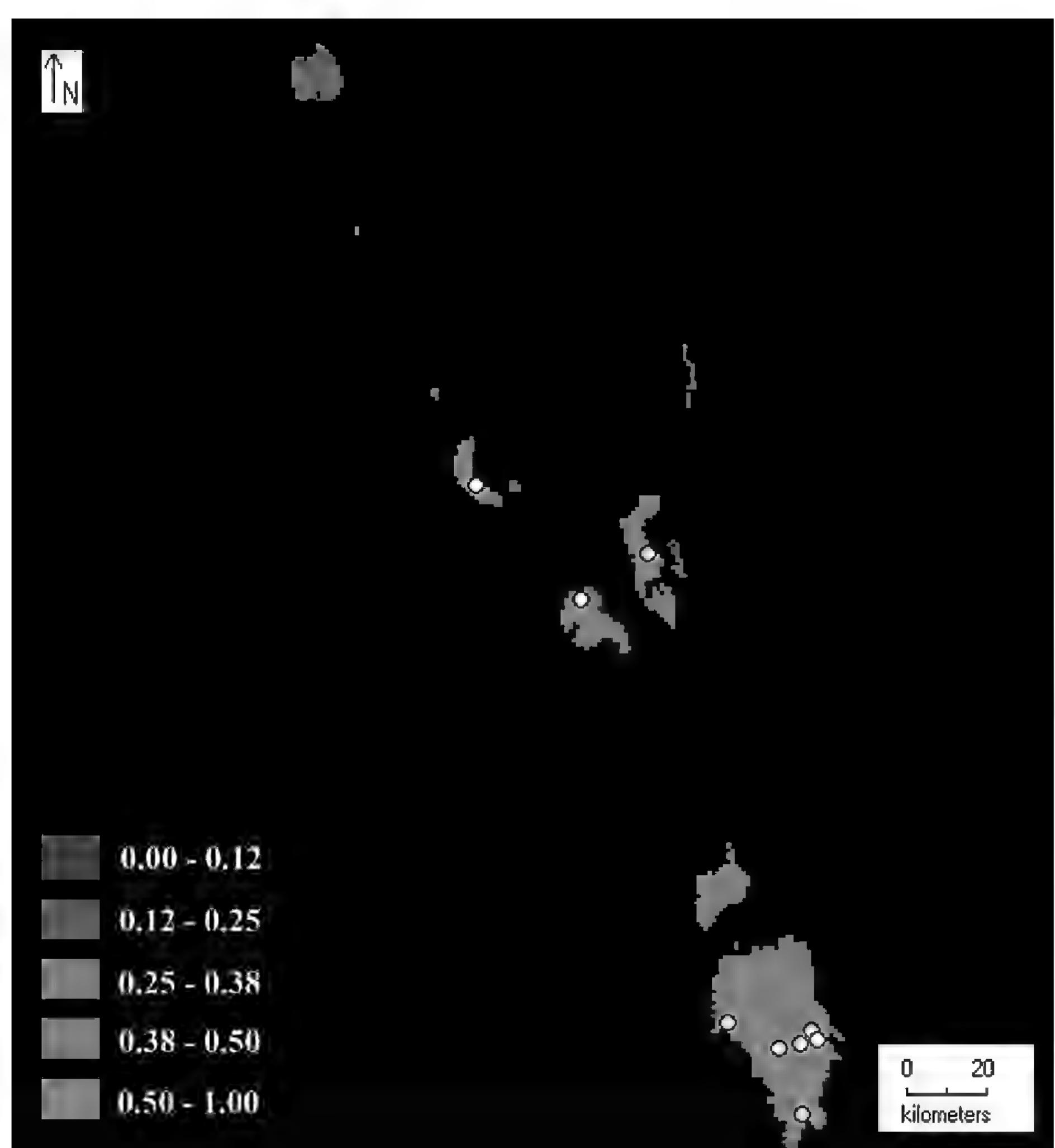
Behavioural observations were made on the recorded individuals of *Dibamus nicobaricum* to observe its pattern of activity in situ. The period of complete exposure of the organism was very meager when compared to the duration spent underground. Whenever the animal ventured under the soil surface, it always reached the bottom of the container, burrowing through the soil nearly 2–3 cm deep and resting underground. It voluntarily ventures out above the soil surface very rarely (during day time). This elusive behavior, its relatively small body size and cryptic colouration could chiefly be the reasons behind the very few records of this species till now. Majority of the local people were not familiar with *Dibamus nicobaricum* and could not recognize it based on the pictures shown.

### (d) Status assessment of *Dibamus nicobaricum*

Based on the projected distribution maps and the number of individuals observed and reported in literature until now, it would be appropriate to regard *Dibamus nicobaricum* as an ‘Endangered’ species as per the norms of the IUCN criteria B1 (Extent of occurrence 3865 km<sup>2</sup>, which is less than 5000 km<sup>2</sup>) and B2 (Area of occupancy estimated to be 36.0 km<sup>2</sup>, which is less than 500 km<sup>2</sup>). However, data on population of *D. nicobaricum* and its fluctuation over a period of time are extremely difficult to establish owing to its rarity, relatively small body size, fossorial behaviour and elusive nature.

## DISCUSSION

*Dibamus nicobaricum* has been recorded only a few times since its original description in 1867. The present study adds to the information on natural history, distribution and behavior of *Dibamus nicobaricum*. Although *Dibamus nicobaricum* was revalidated as a distinct species by Das (1996), Honda et al. (2001) questioned the validity of the Nicobarese species based on the fact



**Fig. 5.** Predicted distribution of *Dibamus nicobaricum* based on occurrence points. Warmer colours denote higher occurrence probability.

that Das (1996) failed to study the holotype housed at the Natural History Museum of Vienna (NMW 23461) and considered it to be a synonym of *Dibamus leucurus* (Bleeker, 1860). In addition, they pointed at certain morphological inconsistencies between the material reported by Das (1996) as *Dibamus nicobaricum* and the holotype that they have examined. Later, Das and Yakoob (2003) argued based on data from the original description of the species by Fitzinger (1867) and reinstated the specific status of *Dibamus nicobaricum*. Originally described as a member of the genus *Rhinophidion*, which is to be treated as a neuter because of its termination “*on*” the specific epithet *nicobaricum* was appropriate which is also in neuter because of its termination “*um*” and the binomial was grammatically correct according to the Art. 31.2 of the ICZN (1999). When Steindachner (1867) referred this species to the genus *Typhloscincus*, he erroneously amended the specific epithet to ‘*nicobaricus*’. Stoliczka (1873), who transferred *nicobaricus* to the genus *Dibamus* from *Typhloscincus* also retained the specific epithet with the masculine termination “*us*”. However, Das (1996) reinstated the correct specific epithet by mentioning this species in the combination *Dibamus nicobaricum* with a masculine generic epithet and a neutral specific epithet, as per the original description. Later, Das (1999) changed it back to the name combination *Dibamus nicobaricus* (sic.) with a masculine generic as well as specific epithet. Likewise, the taxon authorship of *D. nicobaricum* has sometimes been attributed to Steindachner (1867) (e.g., Uetz et al. 2020). However, the cor-

rect representation should be Fitzinger in Steindachner, 1867, because Steindachner (1867: 53) himself credited the description of *Rhinophidion nicobaricum* to Fitzinger by mentioning it as “Fitzinger in lit”.

Though Das (1996) included the Camorta locality in the distribution of the species, he was skeptical about its validity and expressed doubt on its authenticity assuming that the locality could be in error and emphasized the need for its verification. During the present study, *Dibamus nicobaricum* has been recorded from Teressa Island, located further northwest (~ 40 km) of Camorta, which endorses the earlier record from Camorta by Biswas and Sanyal (1980), thereby extending the northern boundary of its distribution range significantly. Likewise, the new record from Galathea Bay in the southern extremity of Great Nicobar Island extends its known distribution range further southwards by at least 30 km. Its confirmed presence in Camorta, Katchall and Teressa Islands in the central group of the Nicobar archipelago make its occurrence on other islands of this group such as Nancowry, Tillanchong and Bompaka highly probable. Also, there is a high probability of its occurrence in the intervening Little Nicobar Island. Records of *D. nicobaricum* have been relatively rare since its description and information on abundance of the species is hard to obtain due to its elusive and fossorial lifestyle. Hence, this species has been considered data deficient by the IUCN until now. Based on the projected distribution maps and the number of individuals observed and reported in literature and the present study, it would be appropriate to regard *Dibamus nicobaricum* as an ‘Endangered’ species as per the norms of the IUCN criteria B1 (Extent of occurrence less than 5000 km<sup>2</sup>) and B2 (Area of occupancy less than 500 km<sup>2</sup>). Several aspects of this species, such as breeding biology, feeding ecology and population estimates still remain unknown. Further studies on such specific aspects would provide us more insights about the endangered and narrowly endemic *Dibamus nicobaricum*.

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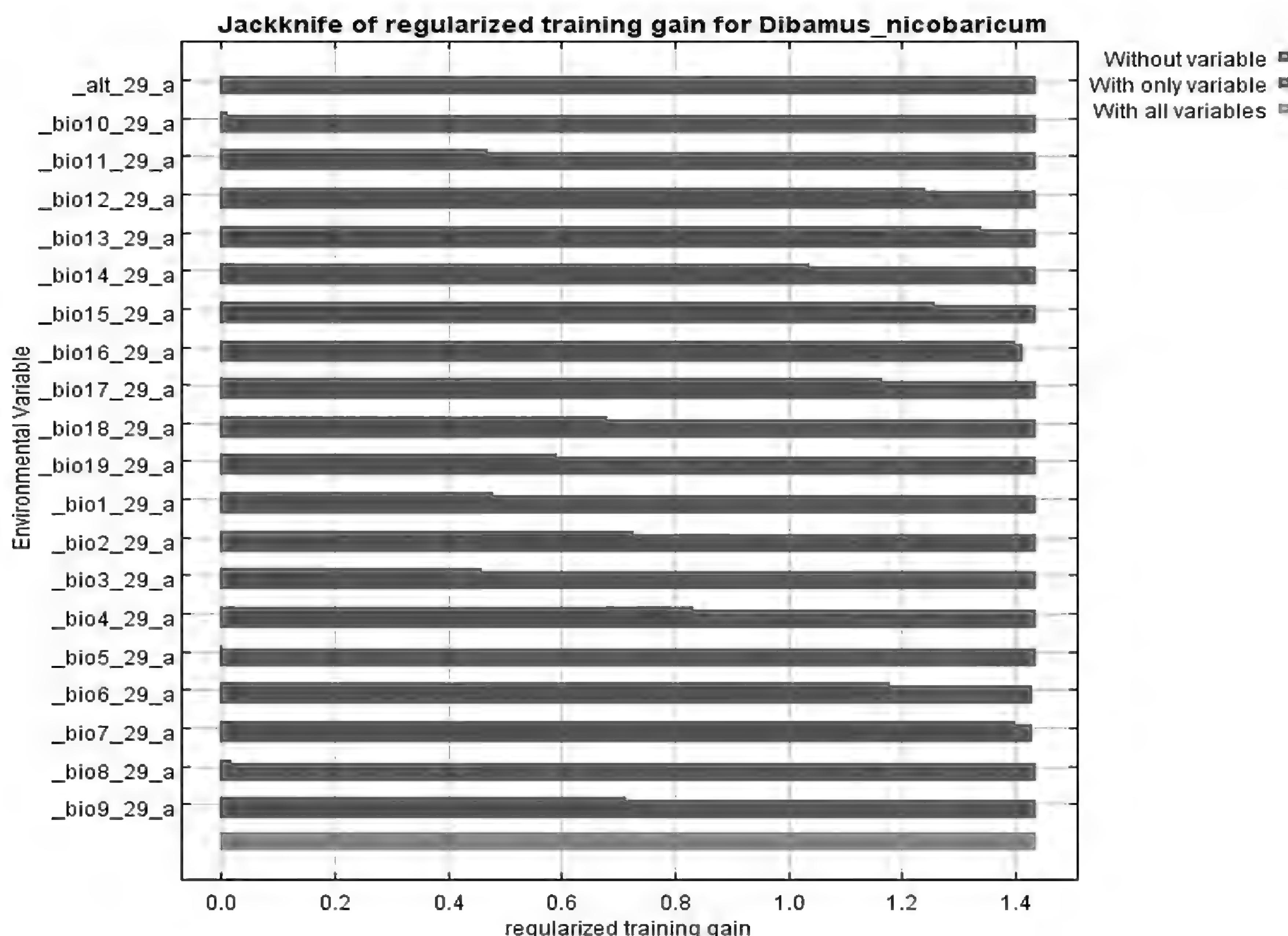


Fig. 6. Jackknife test of variable importance to the model.

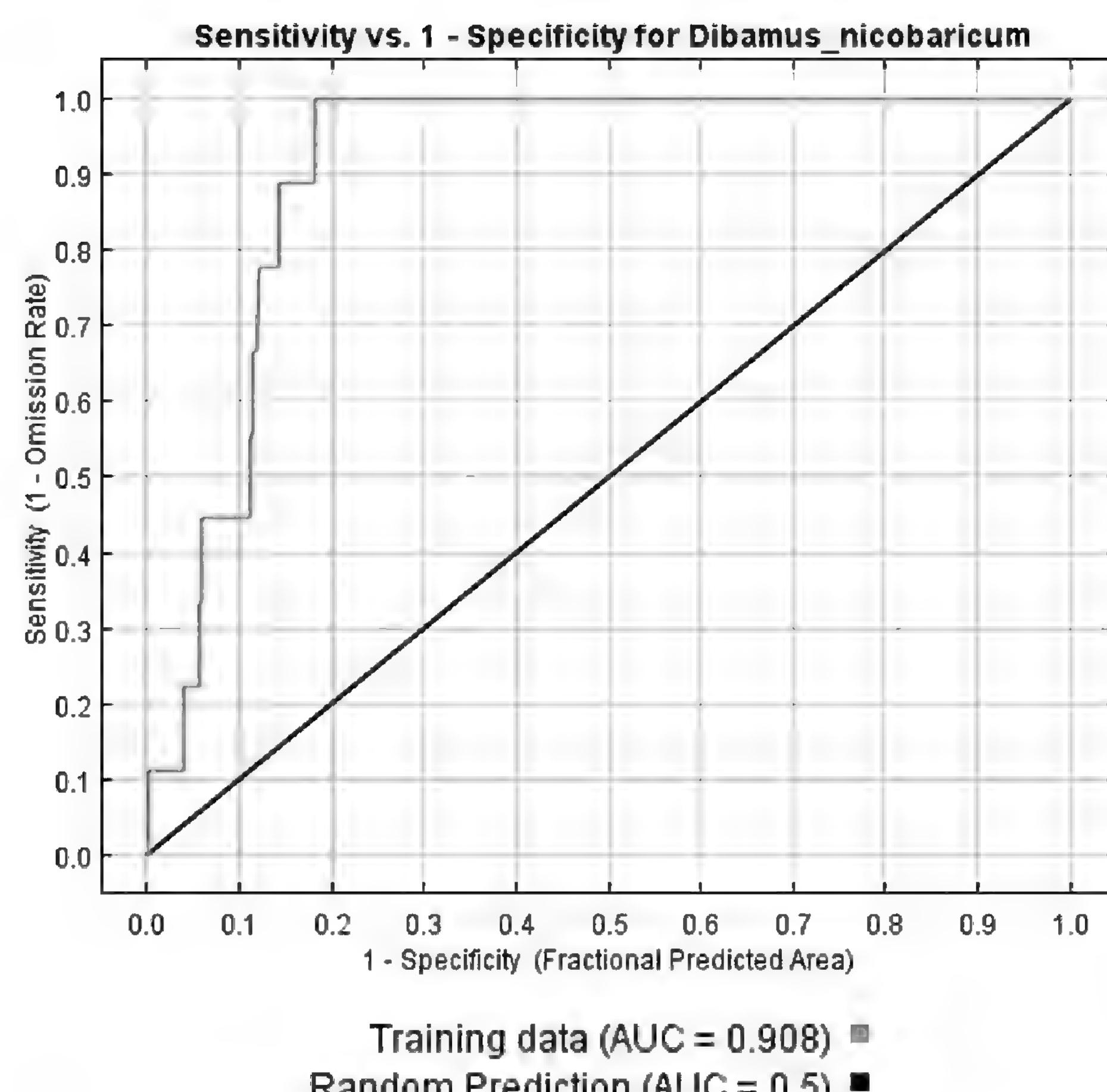


Fig. 7. Receiver operating characteristic curve for the MAX-ENT model predicting the distribution of *Dibamus nicobaricum*.

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## Research article

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# First records of *Chrysotoxum volaticum* Séguay, 1961 from Europe and *Platycheirus marokkanus* Kassebeer, 1998 from Spain (Diptera: Syrphidae) together with additional records of Spanish *Chrysotoxum* Meigen, 1803

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**Abstract.** The first European records of *Chrysotoxum volaticum* Séguay, 1961 from Spain and France, and *Platycheirus marokkanus* Kassebeer, 1998 from Spain are provided. These are further examples of North African species also present in the Iberian Peninsula. Diagnostic characters are given to separate *C. volaticum* and the similar *Chrysotoxum bicinctum* (Linnaeus, 1758), and additional records of other *Chrysotoxum* Meigen, 1803 hoverflies from Spain are also reported. We also provide DNA barcodes for *C. volaticum* and discuss the utility of DNA barcoding to identify species in the genus *Chrysotoxum*.

**Key words.** Ibero-Maghreb fauna, new species records, France, Spain, diagnosis, DNA barcoding.

## INTRODUCTION

Syrphidae is a species rich family of Diptera which has received much attention during the last decades. Adults of this family are often wasp and bee mimic flower visitors, while larvae have very different feeding modes (Rotheray & Gilbert 1999, 2011). Adults of many species are important pollinators (Ssymank & Kearns 2009; Inouye et al. 2015), predatory larvae are important for pest control (Bugg et al. 2008, Pineda & Marcos-García, 2008; Amorós-Jiménez et al. 2012) and saprophagous larvae in the decomposition of organic material (Lardé 1989; Rotheray et al. 2009; Martínez-Falcón et al. 2012). The large amount of knowledge we now have about this family in Europe and the interest syrphids provoke in many academic and applied fields has led the international organisation IUCN to develop a European red list for

Syrphidae (IUCN 2018). The data here reported will help the preparation of a red list from Spain.

## MATERIAL AND METHODS

Morphological terminology follows Thompson (1999). The examined material originates from different collecting events in Spain and France in the last decades. The species were identified using original descriptions (Séguay 1961; Kassebeer 1998) and other literature (Nielsen 1999; de Courcy Williams et al. 2011; Nedeljković et al. 2013; Speight et al. 2013; Young et al. 2016). Many Spanish specimens were collected in the ‘Sierra de Alcaraz’, in the province of Albacete, a low mountainous area with open Pine forests with Mediterranean maquis (Van Steenis et al. 2017). The information provided under

‘Examined material’ is given in the order of region or province, area and locality with an indication of the altitude, collecting date, in which a range is indicated with “–”, the collector, without adding “leg.” in the material studied section, and in some cases a specimen identifying number, rather than the precise label information.

The figures were made by the first author by stacking multiple photos in Zerene Stacker ver. 1.04 and then further edited with GNU Image Manipulation Program ver. 2.8.22. Each individual photo was taken with Cognisys StackShot at fixed intervals with a Canon EOS D6 SLR camera, a Canon MP-E 5× macro-zoom with a Yongnuo YN14EX ring flash attached. The colour plates were made with the aid of a Zeiss camera lucida on a Zeiss-stereomicroscope SV11 by Axel Ssymank.

#### Abbreviations for depositories / collections

AET	= private collection of André van Eck, Tilburg, the Netherlands
ASW	= private collection of Axel Ssymank
CEUA	= Colección Entomológica de la Universidad de Alicante, CIBIO Institute, Alicante, Spain
CPP	= private collection of Chris Palmer, Portsmouth, United Kingdom
DDG	= private collection of Dieter Doczkal, Gaggenau, Germany
JSA	= private collection of Jeroen van Steenis
MNCN	= Museo Nacional de Ciencias Naturales, Madrid, Spain
MNHN	= Muséum National d’Histoire Naturelle, Paris, France
MRL	= private collection of Menno Reemer, Leiden, the Netherlands
MZW	= private collection of Menno van Zuijen
NBC	= Naturalis Biodiversity Center, Leiden, the Netherlands
NHM	= The Natural History Museum, London, UK
SBH	= private collection of Sander Bot, Haren, the Netherlands

The 5' region of the cytochrome *c* oxidase subunit I (COI) gene, the so called DNA barcode (Hebert et al. 2003a, 2003b) was obtained from three specimens of *Chrysotoxum volaticum* Séguy, 1961. Meso- and metalegs from dry, pinned specimens were used for DNA extraction. The extraction protocol follows Mengual et al. (2018) and the specimens were preserved and labelled as DNA voucher specimens for the purpose of morphological studies and deposited at the CEUA. DNA primers and PCR amplification protocols follow Rozo-Lopez & Mengual (2015).

#### RESULTS

Both *Chrysotoxum volaticum* and *Platycheirus marokkanus* have been mentioned to occur in Europe (Ssymank & Doczkal 2007; Van Steenis & Van Steenis 2014), but only records of *P. marokkanus* have been documented so far (Van Eck 2016).

Since Séguy’s original description, *Chrysotoxum volaticum* has been reported only from Morocco by Claussen (1989) and Claussen & Hauser (1990). The original description of *C. volaticum* is not very informative and this species is missing from most identification keys. Thus, we provide here a table (Table 1) of diagnostic characters and a key to distinguish it from the similar *Chrysotoxum bicinctum* (Linnaeus, 1758).

#### *Chrysotoxum bicinctum* (Linnaeus, 1758)

**Distribution.** Widespread throughout Europe, possibly very rare in the Iberian Peninsula. In Spain, records of this species are confirmed just from the provinces of Asturias, Cantabria and Lérida.

**Material examined.** Many records in the authors’ collections throughout Europe. **Spain:** 1♂ (CEUA), Asturias, Santillán, 6-VII-1986, M.A. Marcos García (26b), [det. as *C. bicinctum* form A by A. Ssymank in 2009]; 1♀ (CPP), Cantabria, Potes, Las Ilces, 43°06'41" N, 4°45'27" W, 754 m, 26-VI-2017, C.J. Palmer; 1♀ (CPP), Girona, Setcases, 26-VIII-1996, C.J. Palmer; 1♀ “yellow” (SBH), Lleida, Bordes de Graus, camping, 42°40'07" N 1°14'14" E, 1321 m, 4-VII-2019, S. Bot; 1♀ (SBH), Lleida, near Tavascan, 42°40'59" N, 1°13'59" W, 1400 m, 29-VII-2013, S. Bot; 1♂ (NBC), Lleida, Vall d’Aran, VIII-[19]45; 1♂ (CEUA), Santander, Vada, 22-VI-1987, M.A. Marcos García (34).

The two CEUA males were already published as *C. bicinctum* in Marcos-García (1990) and are here confirmed to belong to this species.

**Remarks.** This species is similar to *Chrysotoxum volaticum* and was, until recently, confused with this species in Europe (Ssymank & Doczkal 2007). Note that there is a form of *C. bicinctum* resembling *C. volaticum* in the more extensively dark coloured wing (Fig. 3F). These specimens are common in the UK and Scandinavia and are generally darker (Figs 2C, 2D), with dark legs, dark abdomen and dark-brown to entirely black (as in Figs 4A, 4C) mouth edge and hypostomal bridge. In the Pyrenees, intermediate forms are also found with a combination of characters in-between *C. bicinctum* and *C. volaticum* indicating possible hybridization.

There is one female (Spain, Pyrenees, coll. SBH) with black mouth edge and hypostomal bridge as in *C. volaticum* and a short wing macula, a black frons with small

pollinose maculae and narrow fascia on tergum IV as in *C. bicinctum*. Two other females (coll. SBH, Spain Lérida and France, Pyrenees) have a yellow hypostomal bridge, a black frons and a short wing macula as in *C. bicinctum*, but large pollinose maculae on the frons and a wide yellow fascia on tergum IV as in *C. volaticum*. These all could be females of *C. bicinctum* in which the last two are “yellow” forms in which the pollinose maculae on the frons are larger. There are three males (France, Pyrenees, coll. SBH), sympatric with one of the “yellow” females, which seem to be more straightforward identified as *Chrysotoxum volaticum*, although the frontal pollinosity, the frontal colour and the colouration of the scutellum seem to be more like in *C. bicinctum*.

### *Chrysotoxum volaticum* Séguy, 1961

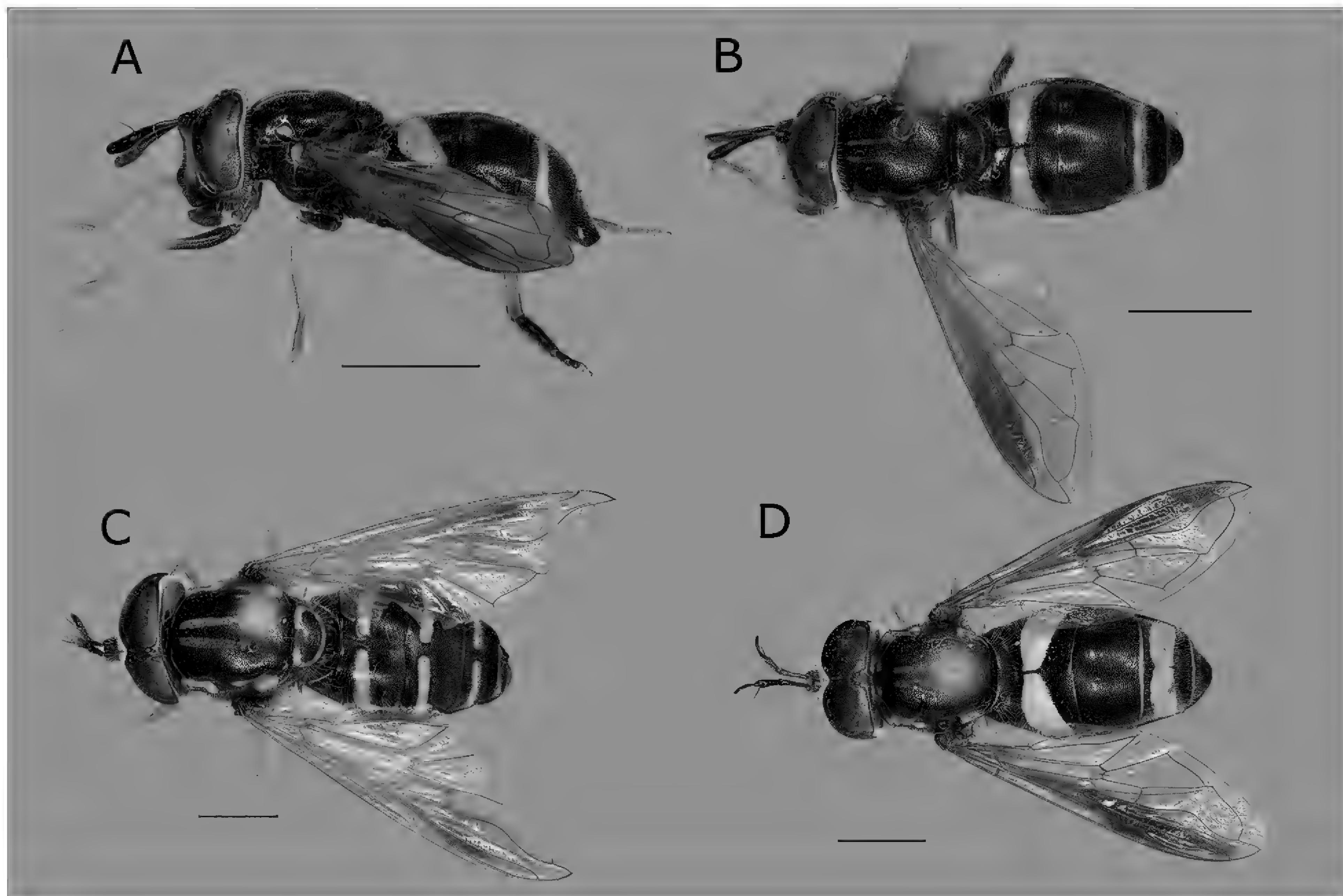
**Distribution.** Originally described from Algeria and Morocco (Séguy, 1961). Recently recorded from Morocco (Claussen, 1989; Claussen & Hauser, 1990). Dirickx (1994) also reported this species in the Mediterranean region of Morocco.

New to Europe and found in France, Portugal and Spain.

**Material examined.** Type series: 2 ♂, 3 ♀, “Moyen Atlas, Ifrane (Morocco), VI-[1]949”, “Museum Paris 1949 L. Chopard”; 1 ♂ “Setif, Algérie, coll. Théry”, “Museum Paris, Algérie, Setif A. Théry 1902” (MNHN). France: 2 ♀ (MRL), Cévennes, Causse Blandas, 10 km SW Le Vigan, 43°54'56" N, 3°28'51" E, 800 m, 20-VIII-2014, M. Reemer; 1 ♀ (JSA), Languedoc-Roussillon, Prades,

**Table 1.** Differentiating characters between *Chrysotoxum volaticum* and *C. bicinctum*.

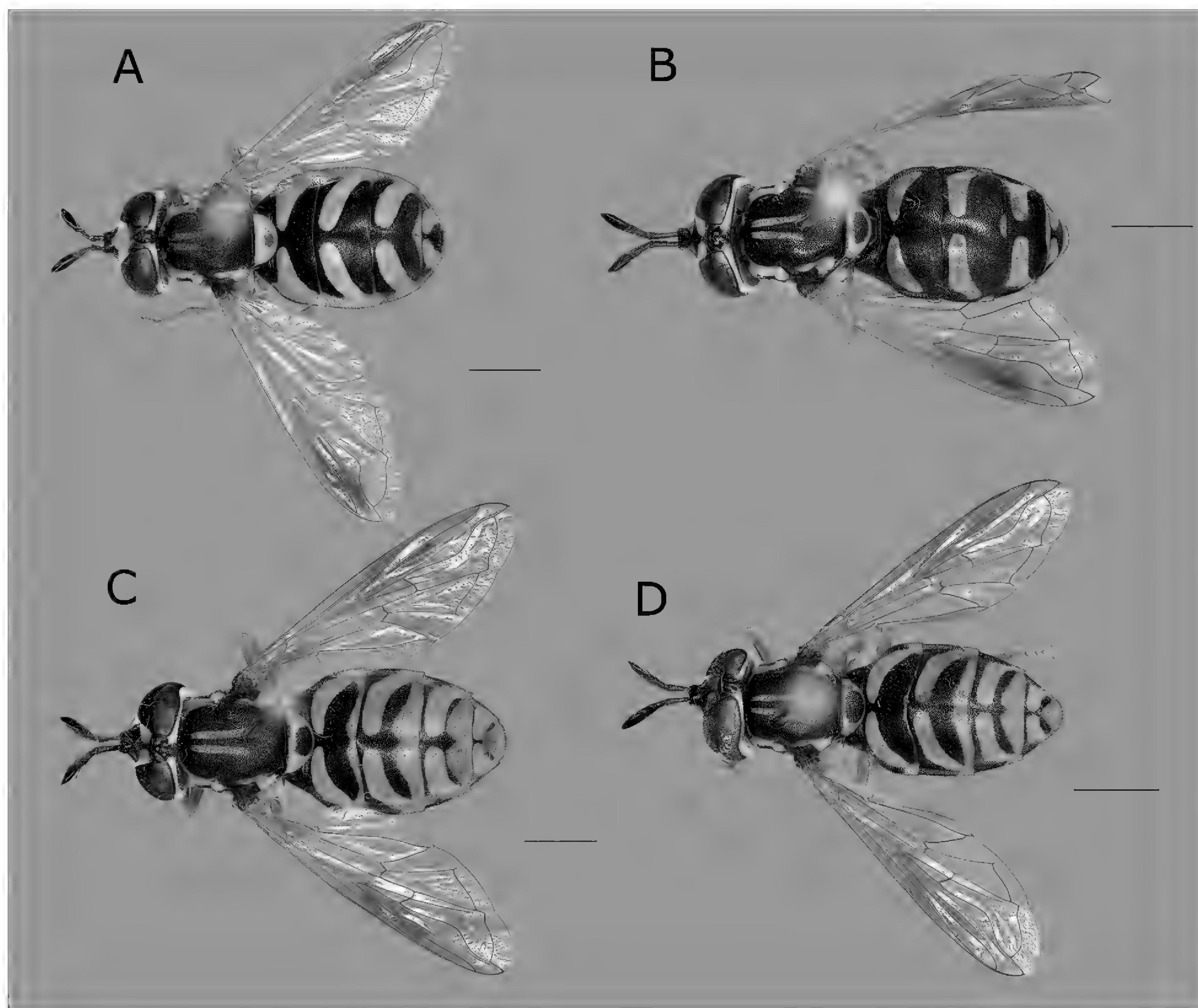
	<i>Chrysotoxum bicinctum</i>	<i>Chrysotoxum volaticum</i>
Colouration of wing	Dark macula on wing margin shorter never reaching wing apex and <b>usually ending as a broad blunt spot above the bow of vein R4+5</b> , usually apical border of dark wing macula clearly demarcated. In dark specimens radial cell R1 also darkened and outer apical border diffuse	Dark brown to black on anterior margin, reaching the wing apex, <b>Radial cell R1 completely darkened and dark patch extending distinctly beyond the tip of this cell, dark spot in cell R2+3 reaching costa for up to about ½ of its length within this cell</b> , wing patch apically and on posterior margin clearly demarcated, sometimes reaching backwards to join R4+5 apically
Scutellum in dorsal view	<b>Almost completely black, with a narrow yellow posterior margin.</b> In bright specimen two narrow yellow or obscured small maculae may be present basally, in males sometimes fused to a narrow basal yellow line	<b>Scutellum largely yellow</b> with scarcely visible narrow black fascia basally and a black median fascia of varying extent: Females usually with broad median black fascia of ca. 0.5–0.7 × of length of scutellum
Frons, colour around the lunulae on frontal prominence	<b>Complete frons dark brown to black;</b> lunulae itself mostly dark brown to black, rarely obscurely yellow or in some specimen yellow	<b>Frons laterally and posteriorly of lunulae yellow.</b> However, the yellow part may be largely obscured with only laterally some dark-yellow colour
Pollinosity on frons	Males: narrow pollinose fascia white Females: <b>pollinose maculae smaller</b> , leaving about 1/5–1/4 of the frons free in the middle	Males: narrow pollinose fascia yellowish Females: <b>pollinose maculae large, almost touching</b> , narrow non-pollinose line in between usually less than 1/5 of width of frons
Ratio: length of eye contiguity/ ocellar triangle (males only)	Eye contiguity often distinctly longer than ocellar triangle, Ratio ca. 1.1–1.3	Almost the same length, Ratio 0.9–1.1
Tergum III	Black usually with very narrow yellow fascia or well developed yellow pair of maculae, however dark specimen have a completely dark tergum III	Black with a very narrow yellow posterior margin, rarely also with small yellow maculae
Tergum IV	<b>Yellow fascia narrow</b> , usually 0.2–0.4 of length of tergum IV, in light specimens wider	<b>Yellow fascia broad</b> , usually 0.4–0.75 of length of tergum IV,
Genae and hypostomal bridge	Genae below eyes with a black vitta, but <b>hypostomal bridge clearly yellow</b> , in dark specimens genae black and hypostomal bridge dark-brown to black	Genae below eyes and hypostomal bridge <b>usually completely black or with dark brownish patches on the sides</b> ; a few individuals may have an almost yellow mouth edge



**Fig. 1.** Adult habitus. **A.** Lateral view. **B–D.** Dorsal view. **A.** *Chrysotoxum bicinctum*, ♀, Sweden. **B.** *C. bicinctum*, ♂, Sweden. **C.** *C. festivum*, ♂, Spain. **D.** *C. volaticum*, ♂, Sierra de Alcaraz. Scale bars: 2.5 mm.

Les Sauterelles, 42°34'15" N 2°24'49" E, 785 m, 12-VIII-2014, J. van Steenis; 1♀ (JSA), Vernet les Bains, Col de Mantet, 42°28'50" N 2°18'53" E, 1765 m, 17-VIII-2014, J. van Steenis; 3♂ (SBH), Pyrenees, Vallée d'Eyne, 42°28'05" N, 2°05'17" E, 1650 m, 23-VII-2013, S. Bot; 1♂ (NBC), Pyrenees Orientales, Font Rocheu, 11-VII-1965, J. v.d Vecht; 1♀ (NBC), Pyrenees Orientales, Fillols, 10-VII-1965, J. v.d. Vecht; 1♀ (NBC), Vaucluse, Carpentras VI-1953, P.M.F. Verhoeff; 12♀ (NBC), Vaucluse, Carpentras, 1–3-VIII-1953, P.M.F. Verhoeff; **Morocco:** 2♀ (NBC), Toubkal Massif, Oukaïmeden, 2500–2800 m, 2–8-VII-1977, v. Oorschot, Houkes & Oosterbroek; 1♀ (NBC), Arhbalou, 43 km S. Marrakech, route S513, 1000 m, 3–14-VII-1977, v. Oorschot, Houkes & Oosterbroek; **Portugal:** 1♀ (NBC), Doure, Resende, 16–19-VII-1953, P.M.F. Verhoeff; 1♀ (AET), Braga, Gilmonde, Barcelos, 41°29'02" N 8°46'00" W, 22-V-2012, R. Andrade; **Spain:** 1♂ (JSA), Albacete, Sierra de Alcaraz, Batán del Puerto, 38°34' N 2°21' W, 1200 m, 21-VI-2003, J. van Steenis; 2♂, 1♀ (JSA), 3♀ (MZW), Sierra de Alcaraz, Puerto de las Crucetillas, 38°31' N 2°26' W, 1400 m, 21-VI-2003, J. van Steenis & M.P. van Zuijen; 1♂, 1♀ (MZW), Sierra de Alcaraz, Puerto de las Crucetillas, 38°32' N, 2°24' W, 1100 m, 21-

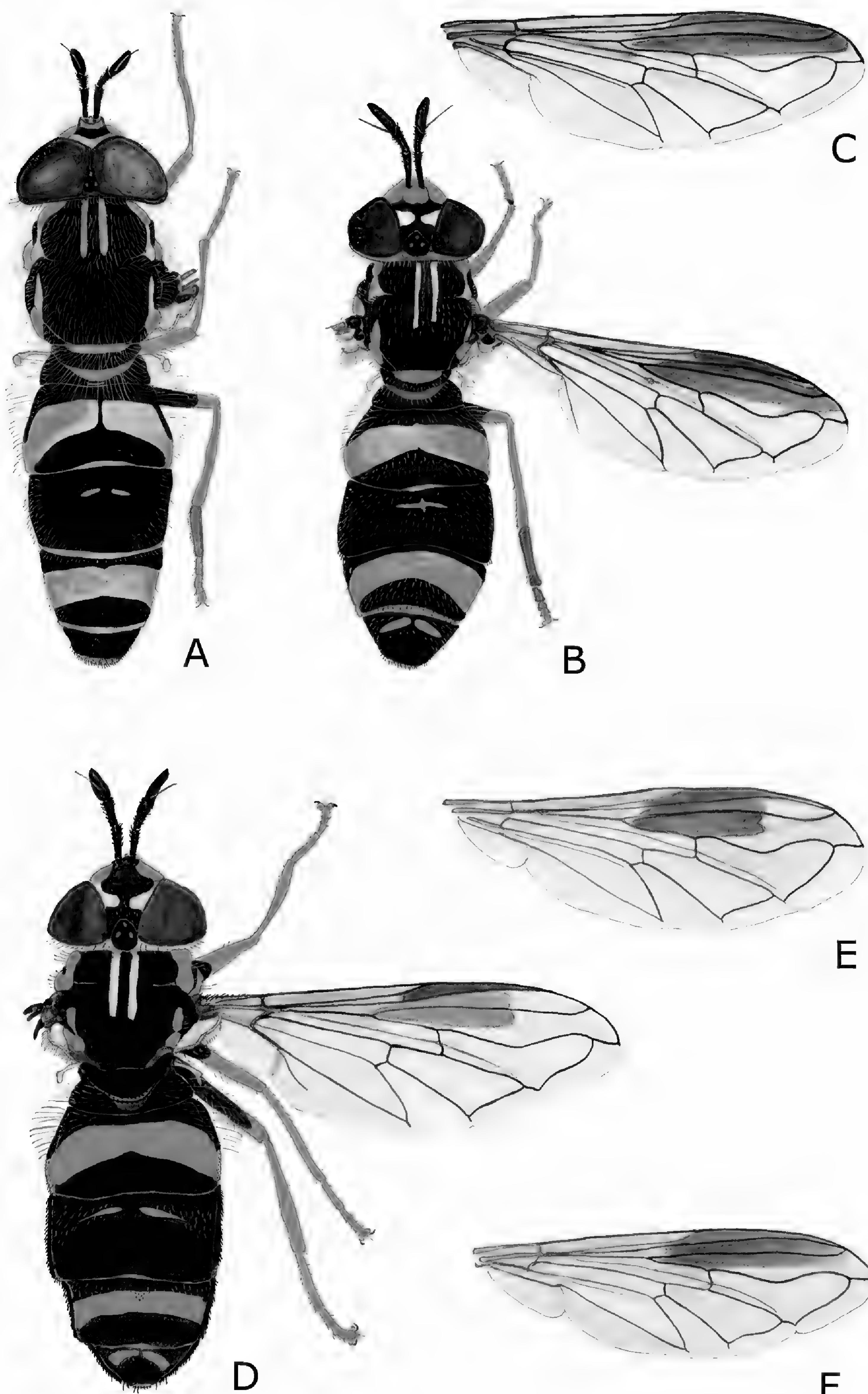
VI-2003, M.P. van Zuijen; 1♂, 1♀ (JSA), 1♀ (MZW), Sierra de Alcaraz, Puerto de las Crucetillas, 38°32' N, 2°23' W, 1000–1200 m, 22-VI-2003, J. van Steenis & M.P. van Zuijen; 1♀ (JSA), Sierra de Alcaraz, Cañada del Provencio, 38°31' N 2°20' W, 1000 m, 22-VI-2003, J. van Steenis; 1♂ (NBC), Andalucía, Prado Llano, 23-VII-1980; 1♀ (NBC), Andalucía, Prado Llano, 24-VII-1980; 1♀ (NBC), Vadillo Castril, Sierra de Cazorla, 3-VIII-1980; 5♂ (MRL), Andalucía, Sierra Nevada, 2 km N of Trevelez, 25-VI-2003, M. Reemer; 1♂ (MRL), Andalucía, Sierra Nevada, Trevélez, 37°00'47" N, 3°15'47" W, 1600 m, 10-VI-2019, M. Reemer; 1♀ (CEUA), Ávila, Becedas, 6-VII-1977, M.A. Marcos-García; 2♂ (ASW), 3♂, 1♀ (DDG), Castilla la Mancha, Sierra de Alcaraz, Riopar, 38°30'17" N, 2°27'36" W, ca. 775 m, 14-VI-2003, A. Ssymank & D. Doczkal; 4♂, 1♀ (ASW), 5♂, 1♀ (DDG), Sierra de Alcaraz, Espinares de León, 38°32'15" N, 2°24'18" W, ca. 1380 m, 14-VI-2003, A. Ssymank & D. Doczkal; 3♀ (CEUA), Ciudad Real, P.N. Cabañeros, respectively 20-V-2005, 5-VII-2005, 24-VIII-2005, A. Ricarte; 3♂ (NBC), Gerona, Ribas de Freser, 900 m, 25-VII-1970, V.S. v.d. Goot; 1♂ (NBC), Gerona, Ribas de Freser, 900 m, 26-VII-1970, V.S. v.d. Goot; 2♂ (NBC), Gerona, Mollo, 1300 m, 17-VII-1970,



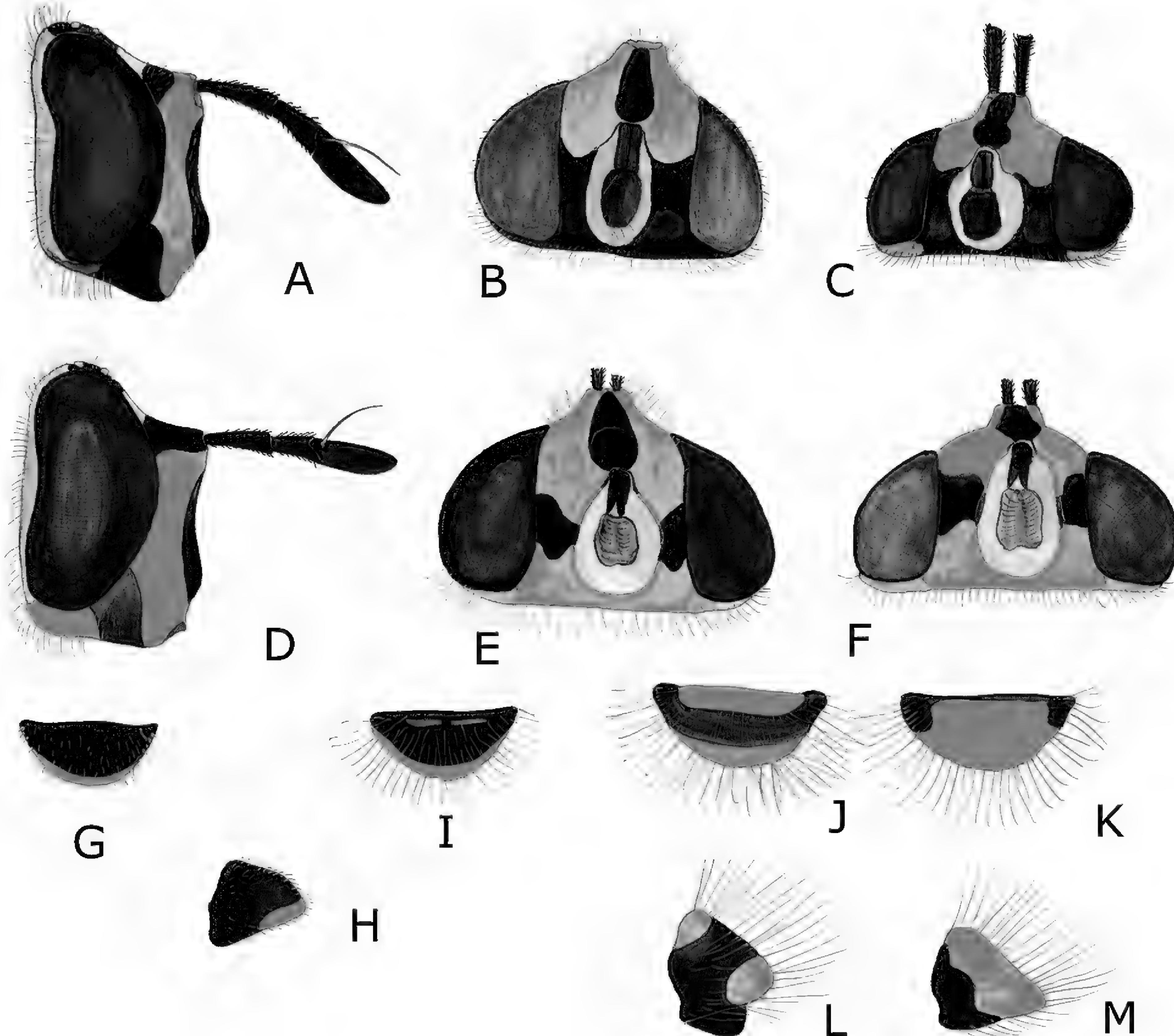
**Fig. 2.** Adult habitus, dorsal view, all from Sierra de Alcaraz. **A.** *Chrysotoxum cisalpinum*, ♀. **B.** *C. gracile*, ♀. **C.** *C. octomaculatum*, ♀. **D.** *C. octomaculatum*, ♀, dark form. Scale bars: 2.5 mm.

V.S. v.d. Goot; 2♂ (NBC), Gerona, Ribas, Zariquey; 1♀ (NBC), La Rioja, dal v Najarilla, S. of Najera, ca 10 km S. of Anguiano, 22-VII–10-VIII-1988, P.J. v. Helsdingen; 2♀ (NBC), Gerona, Ribas de Freser, 900 m, 21-VII-1970, V.S. v.d. Goot; 1♀ (NBC), Gerona, Ribas de Freser, 900 m, 25-VII-1970, V.S. v.d. Goot; 1♀ (NBC), Gerona, Mollo, 1300 m, 19-VII-1970, V.S. v.d. Goot; 1♀ (NBC), Gerona, Sant Ilari de Sacala, VIII-1969, Serra; 1♂ (NBC), Granada, Capileira, 22-VII-1969, H. Overbeek; 1♀ (NBC), Montseny, St Pere de Vilamajor, Sagarra; 2♀ (NBC), Guadalajara, Tierzo bei Mo[illegible]na, 1100 m, 11-VII-1977, W. Schacht; 1♂ (NBC), Guadalajara, Tierzo bei M[illegible], 1100 m, 11-VII-1977, W. Schacht; 2♂ (NBC), Huesca, Torla, 1036 m, 8–26-VII-1974, J. Wolschrijn; 1♀ (MNCN), Huesca, San Juan de la Peña, 1220 m, Exp. Inst. de Entomología, 4-VIII-1943, *Chrysotoxum bicinctum* (L, 1758) Peris Torres det. 1945;

1♂ (MNCN) Jaén, Sierras de Segura, El Pardal, VI-1903, Escalera; 1♂ (CEUA), León, Valdetejas, 1200 m, 13-VII-1977, M.A. Marcos-García; 1♀ (NBC), Lerida, Alamacellas, Zariquey; 1♂ (MNCN), Madrid, Escorial, Colección Lauffer, *Chrysotoxum bicinctum* L, det Strobl; Escorial, VIII-1905, Mercet, *Chrysotoxum bicinctum* L, Gil Collado det; 1♀ (CEUA), Salamanca, Béjar, 5-VII-1978, Carmen Calvo; 3♂ (CEUA), Fresnedoso, 3-VII-1978, Gonzalo Llorente; 1♂ (CEUA), Palomares-Béjar, 10-VII-1978, Gonzalo Llorente; 1♂ (CEUA), Porteros, 14-VI-1981, M. Portillo, *Th. villosa*; 1♂ (CEUA), Vallejosa de Riofrío, 15-VI-1979, Gonzalo Llorente; 1♀ (NBC), Nuria, 1800–2000 m, 22-VII-1970, V.S. v.d. Goot; 1♀ (MNCN), Segovia, San Ildefonso, VII-1906, Escalera; 1♀ (CEUA), Valencia, Chelva, 7–21-VI-1994, C. Pérez-Bañón; 1♀ (CEUA), Requena, 22-VI-1994, C. Pérez-Bañón; 1♂, Utiel, 7-VI-1994, C. Pérez-Bañón;



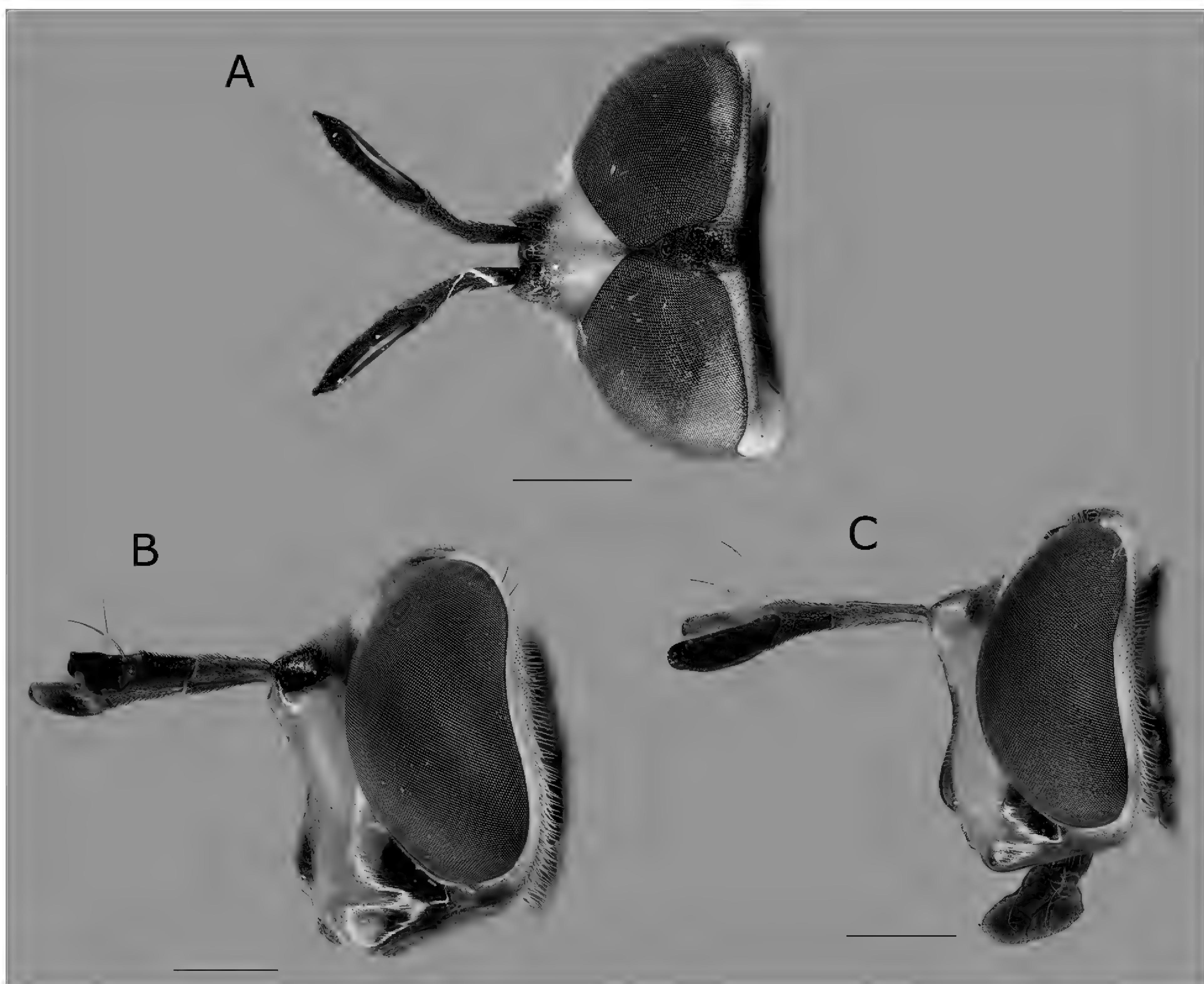
**Fig. 3.** Habitus and wing. **A.** *Chrysotoxum volaticum* ♂. **B.** *C. volaticum* ♀ with wing. **C.** Wing of *C. volaticum* ♂. **D.** *C. bicinctum*, typical form ♀ with wing. **E.** Wing of *C. bicinctum*, typical form ♂. **F.** Wing of *C. bicinctum* form 'A' from Brandenburg, Germany. All drawings ©A. Ssymank.



**Fig. 4.** Details of head and scutellum. **A.** *Chrysotoxum volaticum* ♀, head in lateral view. **B.** *C. volaticum* ♂, head ventral view. **C.** *C. volaticum* ♀ head ventral view. **D.** *C. bicinctum*, typical form ♀, head in lateral view. **E.** *C. bicinctum* ♂ head ventral view. **F.** *C. bicinctum* ♀ head ventral view. **G–H.** *C. bicinctum*, typical form ♀ scutellum, dorsal and lateral view. **I.** *C. bicinctum* form 'A' ♂ scutellum, dorsal view. **J, L, N.** *C. volaticum* ♂ scutellum in dorsal and lateral view, normal colouration. **K, M.** *C. volaticum* ♂ scutellum in dorsal and lateral view, bright colouration. All drawings ©A. Ssymank.

1 ♂ (NBC), Sierra Alta, 1600–1800 m, 26-VII-1965, V.S. v.d. Goot; 1 ♀ (CEUA), Santander, Vada, 22-VI-1987, M.A. Marcos García (34); 1 ♂ (NBC), Tarragona, Prades, 900 m, 1–10-VII-1967, H. & T. v. Oorschot, J. & M. Lourens; 1 ♂ (NBC), Teruel, Sra de Albarracín, Noguera, 1600 m, 12-VII-1977, W. Schacht; 1 ♂ (NBC), Teruel, Sierra de Albarracín, Noguera, 1600 m, 3–6-VIII-1980, W. Schacht; 1 ♀ (NBC), Teruel, Bronemales; 1 ♀ (NBC), Teruel, Aguas Amargas, 1620 m, 21-VII-1965, V.S. v.d. Goot; 3 ♀ (NBC), 24-VII-1965; 2 ♀ (NBC), 29-VII-1965; 1 ♀ (NBC), Teruel, Sierra Alta, 1600–1800 m, 26-VII-1965, V.S. v.d. Goot; 3 ♀ (NBC), Teruel, Pajares, 1000 m, 20-VII-1972, V.S. v.d. Goot; 1 ♀ (NBC),

Teruel, Puerto de Pajares, 1350–1700 m, 21-VII-1972, V.S. v.d. Goot; 3 ♀ (NBC), 22-VII-1972; 1 ♀ (NBC), Teruel, Santa Croche, 1150 m, 5-VIII-1965, V.S. v.d. Goot; 1 ♀ (NBC), Teruel, Santa Croche, 1150 m, 5-VIII-1965, V.S. v.d. Goot; 1 ♀ (NBC), Teruel, Sierra de Albarracín, Noguera, 1600 m, 3–6-VIII-1980, W. Schacht; 2 ♀ (CEUA), Jaén, P.N. Cazorla, Coto Ríos, Llanos de Arance, 38°03'12.1" N, 02°50'10.6" E, 641 m, 13-VI-2019, A. Ricarte (ZFMK-DIP-00067297, ZFMK-DIP-00067298); 1 ♂ (CEUA), Jaén, Coto Ríos, meadow in riverbank opposite Fuente de la Pascuala camping site, 38°03'13" N, 2°50'09" E, 641 m, 29-V-2018, E. Galante (ZFMK-DIP-00067299).



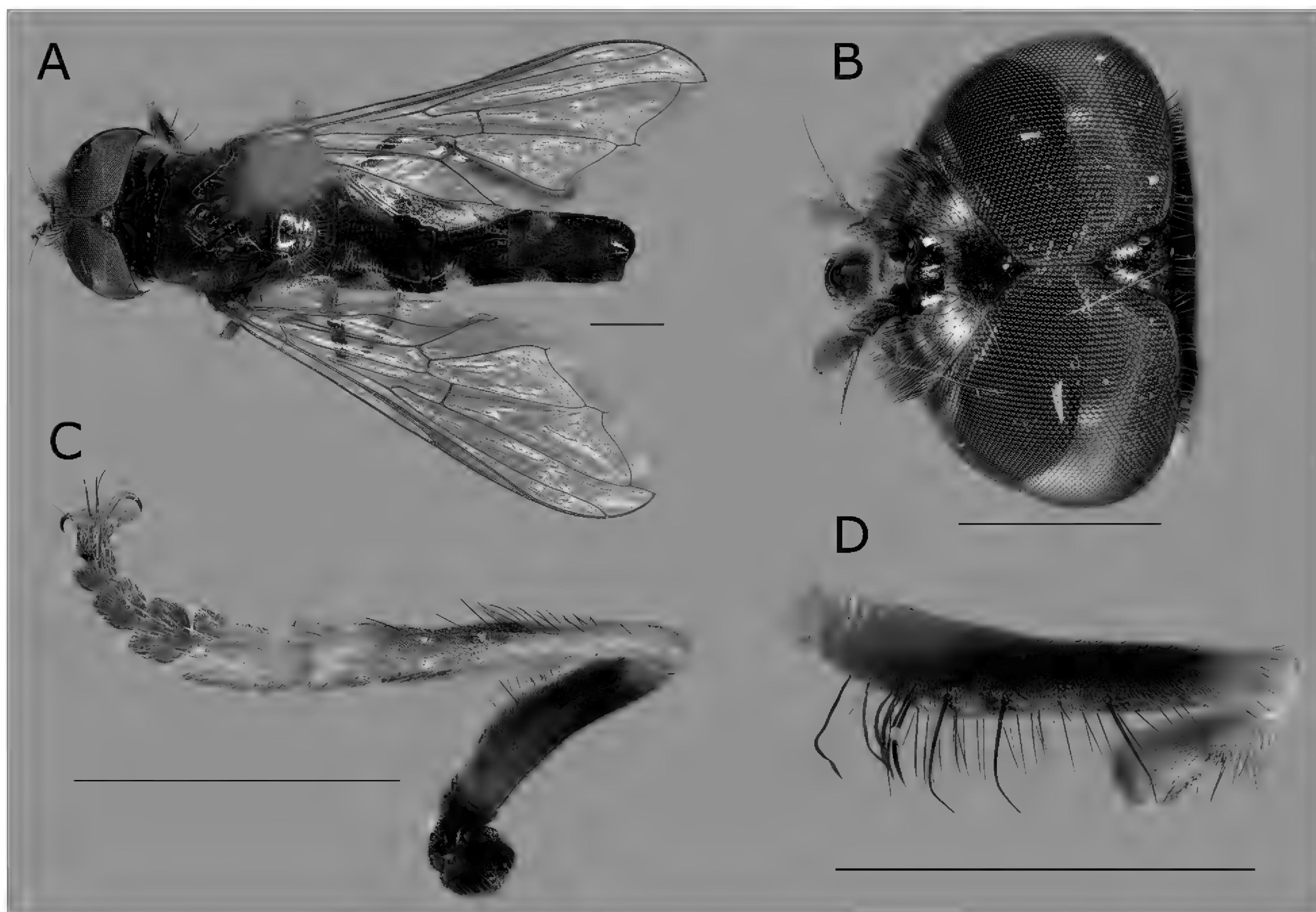
**Fig. 5.** Head; **A.** Dorsal view. **B–D.** Lateral view. **A.** *Chrysotoxum cisalpinum*, ♂, Sierra de Alcaraz. **B.** *C. festivum*, ♀, Spain. **C.** *C. volaticum*, ♀, Sierra de Alcaraz. Scale bars: 1.0 mm.

**Remarks.** This species is similar to *Chrysotoxum bicinctum*, differing by the characters mentioned in Table 1. See also Figs 3A–C, 4A–C, 4J–M.

Many Spanish specimens were collected while visiting flowers of *Thapsia villosa* L.

The Spanish examined material represents the first record from Spain, including specimens previously identified as *C. bicinctum* from the provinces of Jaén, Madrid, Segovia (Gil Collado, 1930), Ávila, Cáceres, Salamanca (Marcos-García 1986), Valencia (Pérez-Bañón, 1995), Ciudad Real (Ricarte 2008) and Huesca (MNCN specimen identified as *C. bicinctum* by Peris Torres), all of them now confirmed to belong to the species *C. volaticum*. All other records of *C. bicinctum* in Ricarte & Marcos-García (2017), which were not accessible to the authors of the present paper, must be regarded as doubtful until they are re-examined.

**Genetics.** Three specimens were successfully sequenced and the 5'-COI sequences were submitted to GenBank (accession numbers MT517826, MT517825, MT517824 for specimens ZFMK-DIP-00067297, ...00067298, and ...00067299 respectively). The obtained three DNA barcodes are identical. We compared our molecular data with two other DNA barcodes from specimens identified as *C. volaticum* from Morocco (Jeff H. Skevington, unpub. data), and the two Moroccan specimens have an uncorrected pairwise-distance of 0.017–0.0185 (similarity of 98.3% and 98.15% respectively) with our specimens, and the uncorrected pairwise-distance between them is 0.00155 (99.845% similarity). The Barcode Index Number (BIN) (Ratnasingham & Hebert 2013) helps to assign individuals to presumptive species, called operational taxonomic units (OTUs) and they are good estimators of valid species, but there are cases of discordance between BINs and accepted species boundaries. For *C. vol-*



**Fig. 6.** *Platycheirus marokkanus*, ♂, from Sierra de Alcaraz. **A.** Adult habitus, dorsal view. **B.** Head, dorsal view. **C.** Protibia and tarsus dorsal view. **D.** Profemur, dorsal view. Scale bars: 1.0 mm.

*aticum* there is the BIN BOLD:AAJ0967 (<https://doi.org/10.5883/BOLD:AAJ0967>), but individuals assigned to this BIN were identified to belong to 12 different morphological species, e.g., *Chrysotoxum arcuatum* (Linnaeus, 1758), *C. bicinctum*, *C. biguttatum* Matsumura, 1911, *C. elegans* Loew, 1841, *C. festivum* (Linnaeus, 1758), *C. graciosum* Violovitsh, 1975, *C. montanum* Nedeljković & Vujić, 2015, *C. octomaculatum* Curtis, 1837, *C. sapporense* Matsumura, 1916, *C. vernale* Loew, 1841, *C. verralli* Collin, 1940, and *C. volaticum*. This means that the algorithm that automatically assigns individuals to OTUs based on sequence variation in the COI DNA barcode region is not able to find enough differences among the COI sequences of these species; in other words, the intraspecific variation of this gene region overlaps with the interspecific variation among these 12 species.

The nearest-neighbour in BOLD systems (<http://www.boldsystems.org>) is *C. orthostylum* Nedeljković & Vujić, 2015 (with BIN BOLD:ADJ6446), but the p-distance within BOLD:AAJ0967 overlaps with the distance between the two BINs: in other words, there is no barcod-

ing gap between the members of BOLD:AAJ0967 and *C. orthostylum*.

#### Modification to existing keys

In the current identification keys (e.g., Speight et al. 2016; Bot & Van de Meutter, 2019) the following text should be added to separate *Chrysotoxum bicinctum* from *C. volaticum*:

5. Yellow fascia on tergum III as deep as those on tergum IV ..... 6
- Yellow fascia on tergum III strongly reduced, absent to at most half the width of the fascia on tergum IV ..... 5A
- 5A. Wing with large dark macula; radial cell  $R_1$  completely darkened (usually blackish) and dark macula extending distinctly beyond the apex of this cell, almost reaching the apex of the wing and reaching costal border on about  $\frac{1}{2}$  of its length within cell  $R_{2+3}$ , dark wing macula usually apically clearly demarcated; scutellum almost completely yellow or with a dark-brown to black median fascia,

at least basally in dorsal view broadly yellow; frontal prominence immediately above insertion of antennae yellow to brown-yellow, in some specimens blackish; genae below eyes and hypostomal bridge usually completely black or with dark brownish maculae on the sides, rarely partly yellow .....  
 ..... *Chrysotoxum volaticum* Séguy, 1961

— Dark macula on wing margin shorter, never reaching wing apex and usually leaving the apical area of the radial cell  $R_1$  clear, if extending beyond the apex of  $R_1$  not reaching the costal border of cell  $R_{2+3}$ ; scutellum black with at most apex narrowly yellow, sometimes with small yellow macula or narrow yellow fascia, anterior part black (in extensively yellow coloured specimens sometimes also a yellow fascia along the anterior margin); frons immediately above lunule black to blackish-brown; genae below eyes with a black fascia and hypostomal bridge yellow, in dark specimens genae entirely black and hypostomal bridge brownish-black .....  
 ..... *Chrysotoxum bicinctum* (Linnaeus, 1758)

#### Additional records of species of *Chrysotoxum* from Spain

##### *Chrysotoxum cautum* (Harris, 1776)

**Distribution.** Widespread throughout Europe. Recorded from several Spanish provinces, from Alicante to Lugo (Ricarte & Marcos-García, 2017).

**Examined material. Spain:** 4♂, 5♀ (MZW), Zaragoza, Hoya de Huesca county, Murillo de Gállego, 1-V-2010, M.P. van Zuijen.

**Remarks.** This is an easily recognizable species based on the extensively yellow abdomen, the large male genitalia and the cleft in female tergum V. This is the first record for the province of Zaragoza.

##### *Chrysotoxum cisalpinum* Rondani, 1845

**Distribution.** Widespread in the Mediterranean region. In Spain, recorded only from the provinces of Madrid and Salamanca (Ricarte & Marcos-García, 2017; Ricarte et al. 2018).

**Examined material. Spain:** 1♂ (JSA), Albacete, Sierra de Alcaraz, Batán del Puerto, 38°34' N 2°21' W, 1200 m, 21-VI-2003, J. van Steenis; 5♂, 4♀ (JSA), 4♀ (MZW), Sierra de Alcaraz, Puerto de las Crucetillas, 38°31' N 2°26' W, 1400 m, 21-VI-2003, J. van Steenis & M.P. van Zuijen; 2♂, 1♀ (JSA), Sierra de Alcaraz, Puerto de las Crucetillas, 38°32' N 2°24' W, 1200 m, 21-VI-2003, J. van Steenis; 2♂, 1♀ (JSA), 1♂ (MZW), Sierra de Alcaraz, Puerto de las Crucetillas, 38°32' N 2°23' W, 1000-

1200 m, 22-VI-2003, J. van Steenis & M.P. van Zuijen; 1♂ (DDG), Riópar, Sierra de Alcaraz, 38°30'17" N, 2°27'36" W, 750 m, 14-VI-2003, D. Doczkal; 4♀ (ASW), Espineras de León, Sierra de Alcaraz, close to Río de los Endrinas, 38°32'15" N, 2°24'18" W, 1380 m, 14-VI-2003, A. Ssymank.

**Remarks.** This species is easy to recognize based on the predominantly yellow frons (Figs 1A, 5A). Most of the specimens were collected on flowers of *Thapsia villosa*. This is the first record for the province of Albacete.

##### *Chrysotoxum festivum* Linnaeus, 1758

**Distribution.** A widespread species in the Palaearctic region. In Spain recorded from several provinces in the northern half (Ricarte & Marcos-García, 2017).

**Examined material. Spain:** 1♂, 4♀ (JSA), Lleida, Sort, Col del Canto, 38°22'17" N 1°14'10" E, 1725 m, 18-VIII-2014, J. van Steenis.

**Remarks.** These specimens (Fig. 1B) differ from central European specimens of *Chrysotoxum festivum* by the entirely black hypostomal bridge (Fig. 5B); sternum II entirely black, anteriorly with yellow macula in *C. festivum*; black facial vitta very broad, broader than in other specimens; wing extensively bare of microtrichia, at most along anterior margin of cell Cu narrowly bare in *C. festivum*. Further Iberian material and a study of relevant type species is needed to establish the true identity of these specimens. This is the first record for the province of Lleida.

##### *Chrysotoxum gracile* Becker, 1921

**Distribution.** Spain and France (Becker, 1921; Speight et al., 2013). In Spain, recorded only from the provinces of Avila, Huesca and Madrid (Ricarte & Marcos-García, 2017).

**Examined material. Spain:** 3♂, 1♀ (MZW), Albacete, Sierra de Alcaraz, Puerto de las Crucetillas, 38°32' N 2°24' W, 1100 m, 21-VI-2003, M.P. van Zuijen; 1♀ (JSA), Albacete, Sierra de Alcaraz, Puerto de las Crucetillas, 38°31' N 2°26' W, 1400 m, 21-VI-2003, J. van Steenis; 3♂ (CPP), Cantabria, Potes, Las Ilces, 43°06'41" N, 4°45'27" W, 754 m, 26-VI-2017, CJ Palmer; 1♀ (CEUA), Madrid, Universidad Rey Don Juan Carlos, en *Sil. rupestris*, 21-VII-2011, *Chrysotoxum festivum*? det. M<sup>a</sup>A. Marcos, *Chrysotoxum gracile* det. Ricarte and Nedeljković X-2019 (103); 2♂ (CPP), Palencia, Alto de la Varga, 42°54'32" N, 4°38'33" W, 1404 m, 24-VI-2017, CJ Palmer.

**Remarks.** This species (Fig. 1C) can be identified using the key in Speight et al. (2016). The narrower abdomen and the bicoloured metafemur, although sometimes very vague, are good characters to separate this species from *Chrysotoxum festivum* in which the abdomen is wider and the metafemur is almost entirely darkish orange-yellow, strongly contrasting with the yellow metatibia. The specimens from Sierra de Alcaraz were found visiting flowers of *Thapsia villosa*, while that from Madrid was found in flowers of *Silene rupestris* L. This is the first record for the province of Albacete.

***Chrysotoxum octomaculatum* Curtis, 1837**

**Distribution.** Widespread in Central and Southern Europe. In Spain, scattered records from Alicante to Pontevedra (Ricarte & Marcos-García, 2017).

**Examined material. Spain:** 2♀ (JSA), Albacete, Sierra de Alcaraz, Batán del Puerto, 38°34' N 2°21' W, 1200 m, 21-VI-2003, J. van Steenis; 1♂ (MZW), Sierra de Alcaraz, Puerto de las Crucetillas, 38°32' N 2°24' W, 1100 m, 21-VI-2003, M.P. van Zuijen; 1♀ (JSA), 1♀ (MZW), Sierra de Alcaraz Puerto de las Crucetillas, 38°31' N 2°26' W, 1400 m, 21-VI-2003, J. van Steenis & M.P. van Zuijen; 2♂, 3♀ (JSA), 1♀ (MZW), Sierra de Alcaraz, Puerto de las Crucetillas, 38°32' N 2°23' W, 1000-1200 m, 22-VI-2003, J. van Steenis & M.P. van Zuijen; 1♂, 1♀ (ASW), 2♂ (DDG), Riópar, Sierra de Alcaraz, 38°30'17"N, 2°27'36"W, 775 m, 14-VI-2003, A. Ssymank & D. Doczkal; 2♂ (ASW), 3♂, 2♀ (DDG), Espineras del León, Sierra de Alcaraz, close to Río de los Endrinalles, 38°32'15"N, 2°24'18"W, 1380 m, 14-VI-2003, A. Ssymank & D. Doczkal; 1♂ (CPP), Cantabria, Potes, Las Ilces, 43°06'41"N 4°45'27"W, 26-VI-2017, 754 m, C.J. Palmer; 6♂, 1♀ (ASW), 8♂ (DDG), Granada, Cortijo los Capotes, Almijara, 36°52'44"N, 3°43'54"W, 1240-1270 m, 11-VI-2003, A. Ssymank & D. Doczkal; 2♂ (ASW), 1♂ (DDG), Bosque del Puerto Navazo, Alhama de Granada, *Quercus rotundifolia*-forest, 36°58'29"N, 4°00'59"W, 1180 m, 12-VI-2003, A. Ssymank & D. Doczkal; 1♀ (DDG), Jaén, Sierra Morena, Río Guarizas, 36°24'27"N 3°23'00"W, 790 m, 13-VI-2003, D. Doczkal; 3♂ (CPP), Palencia, Cervera de Pisuerga, 42°52'24"N 4°31'17">W, 1144 m, 23-VI-2017, C.J. Palmer.

**Remarks.** This is a large and extensively yellow coloured species, especially in southern populations (Fig. 2C), although two smaller and rather dark specimens were collected (Fig. 2D), somewhat resembling *Chrysotoxum verralli* Collin, 1940. Most of the specimens were collected while visiting flowers of *Thapsia villosa*. These are the first record for the province of Albacete, Cantabria, Granada and Jaén.

**Records of species of *Platycheirus marokkanus* from Spain**

***Platycheirus marokkanus* Kassebeer, 1998**

**Distribution.** Morocco and Portugal (Kassebeer, 1998; van Eck, 2016). New to Spain.

**Examined material. Spain:** ♂ (JSA), Albacete, Sierra de Alcaraz, Batán del Puerto, 38°34' N 2°21' W, 1200 m, 21-VI-2003, J. van Steenis; 1♂ (DDG), Granada, Almijara, Cortijo los Capotes, 36°52'44"N, 3°43'54"W, 1240-1270 m, 11-VI-2003, D. Doczkal.

**Remarks.** This species (Fig. 6) belongs to the *Platycheirus albimanus* sub-group and is most similar to *P. laskai* Nielsen, 1999. In *P. albimanus* (Fabricius, 1781), *P. ciliatus* Bigot, 1884 and *P. muelleri* Marcuzzi, 1941 the apex of the protibia and the first tarsomere of protarsus is much wider than in *P. marokkanus*, and in *P. nigrofemoratus* Kanervo, 1934 the first tarsomere of protarsus is clearly wider than the maximum width of the protibia. In *P. laskai*, *P. marokkanus* and *P. nigrofemoratus* the setae on the protibia are short, at most as long as medial width of protibia, while in the other species these setae are long, more than twice as long as medial width of protibia. The angle of approximation of the eyes in males is 90° in *P. albimanus*, *P. laskai* and *P. nigrofemoratus*; about 100° in *P. marokkanus*; and about 120° in *P. ciliatus* and *P. muelleri*. Two species each have one character not shared with any other species; *P. laskai* has only one long black seta on profemur, which is placed apically, all others have three setae placed baso-medially; *P. ciliatus* has the basal tuft of setae on profemur with slightly flattened apex, while in all others the apex of these setae is narrowly rounded. In *P. muelleri* the ventral surface of protarsus has characteristic black markings, while in *P. marokkanus* these markings are reduced and at most dark-brownish.

The male specimen from Albacete was collected on flowers of *Thapsia villosa*.

**DISCUSSION**

The genus *Chrysotoxum* has been studied intensively during the past decade and several cryptic species have been described (e.g., Nedeljković et al. 2013, 2015; Vujić et al. 2017), with focus on the Balkan Peninsula and the Middle East fauna. These studies revealed a reality that some taxonomists knew beforehand (e.g., Sommaggio 2001): there are species complexes within *Chrysotoxum*. The genus shows large inter- and intraspecific variability in adult characters and an almost lack of species specific characters in genital morphology (Sommaggio 2001; Nedeljković et al. 2013) and the use of molecular data

showed a similar degree of variability (Masetti et al. 2006; Nedeljković et al. 2015), a fact corroborated with our new DNA barcodes. The material of *Chrysotoxum volaticum* collected by the authors in Spain was compared to the type material of Séguy in the National History Museum in Paris (Muséum Nationale d'Histoire Naturelle) and can be clearly assigned to *Chrysotoxum volaticum* Séguy, 1961. The validity of the species *C. volaticum* appears to be confirmed not only by morphology but also by the simultaneous co-occurrence of this species and *C. bicinctum* in the Spanish and French Pyrenees, plus the presence of several intermediate specimens. The majority of specimens of *Chrysotoxum bicinctum* and *C. volaticum* can be identified without doubt. Only in a few cases yellow forms of *C. bicinctum* could be identified as *C. volaticum* and dark forms of *C. volaticum* could be identified as *C. bicinctum*.

Species identification using molecular characters has been argued as an application of DNA barcoding (Hebert et al. 2003a, 2003b). The use of DNA barcodes to distinguish species is based on the so-called “barcoding gap” (Meyer & Paulay 2005), in other words, that the intraspecific genetic distance of one species is much less than the interspecific distance between this species and its closest relative. The published literature is full of examples where there is no barcode gap between a group of species (e.g., Wiemers & Fiedler 2007; Robinson et al. 2009; van Velzen et al. 2012; Koroiva & Kvist 2017). It seems that the geographical scale is one of the reasons that explains the low or high intraspecific variation of the COI gene (Bergsten et al. 2012), which strongly depends on taxonomic groups and practices (Čandek & Kuntner 2015). In the family Syrphidae, some published datasets already point out that the use of COI as DNA barcode does not help in species identification as the barcoding gap does not exist in certain species groups or genera, e.g., Mengual et al. (2006) and Ståhls et al. (2009) for *Merodon* Meigen, 1803, Haarto and Ståhls (2014) for *Melanostoma* Schiner, 1860, Jordans et al. (2015) for some Afrotropical species and genera, Nedeljković et al. (2018) for *Xanthogramma* Schiner, 1860, Morinière et al. (2019) for some species of the German hoverfly fauna, among others. Masetti et al. (2006) were the first to report very low interspecific molecular divergence among *Chrysotoxum* species. Skevington and Sommaggio (2009) pointed out this problem for the Nearctic species of *Chrysotoxum*. The lack of resolution when using DNA barcoding in *Chrysotoxum* was again stated for the Palaearctic species in Nedeljković et al. (2013) and from the results of large barcoding campaigns, such as “German Barcode of Life” (GBOL, [www.bolgermany.de](http://www.bolgermany.de); Geiger et al. 2016) and “Barcoding Fauna Bavarica” (BFB, [www.faunabavarica.de](http://www.faunabavarica.de); Haszprunar 2009). Our new DNA barcodes of *C. volaticum* are identical to sequences of *C. festivum* and *C. bicinctum*, and are very similar (>99%) with other *Chrysotoxum* species of the BIN BOLD:AAJ0967. New

molecular markers need to be studied to prove their suitability for species identification in the genus *Chrysotoxum*, but so far, the 5' region of the COI gene performs very poorly.

The habitat in the Sierra de Alcaraz where *Chrysotoxum volaticum* was collected, with grassy vegetation in the vicinity of a brook in a valley at high altitude in a mountain range, is comparable to the situation described by Claussen & Hauser (1990) for the localities from the Middle Atlas Mountains in Morocco. All other specimens from central and northern Spain were mostly collected in *Quercus pyrenaica* Willd. forests and, in the case of the specimens from Cabañeros N.P., the sampling locality had forest with a peat-bog and a temporal stream. In Spain, adults of *C. volaticum* were observed on flowers of *Thapsia villosa* L. (yellow-flowering tall Apiaceae) which were in full flower. *Thapsia villosa*, together with other plants, was also recorded as a visited flower by Marcos-García (1986). Many individuals were observed on the flowers, amongst other species of *Chrysotoxum*, wasps and other Diptera. These are the first data about flowers visited by *C. volaticum*.

Although some specimens have overlapping characters of *C. volaticum* and *C. bicinctum*, see under the remarks section of *C. bicinctum*, most studied specimen can be assigned clearly to either one. More specimens from other parts of their distributional range need to be studied, especially the intermediate forms from the Pyrenees, in order to corroborate the validity of the morphological characters used here to separate these two species and to define better the species within this species group. This is part of ongoing research to study the species of the genus *Chrysotoxum* (Ricarte et al. 2019). The Ibero-Maghreb *Chrysotoxum* fauna is in need of a thorough revision. Hopefully though, this paper will aid in the recognition of these species as faunistic elements of the Iberian Peninsula.

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